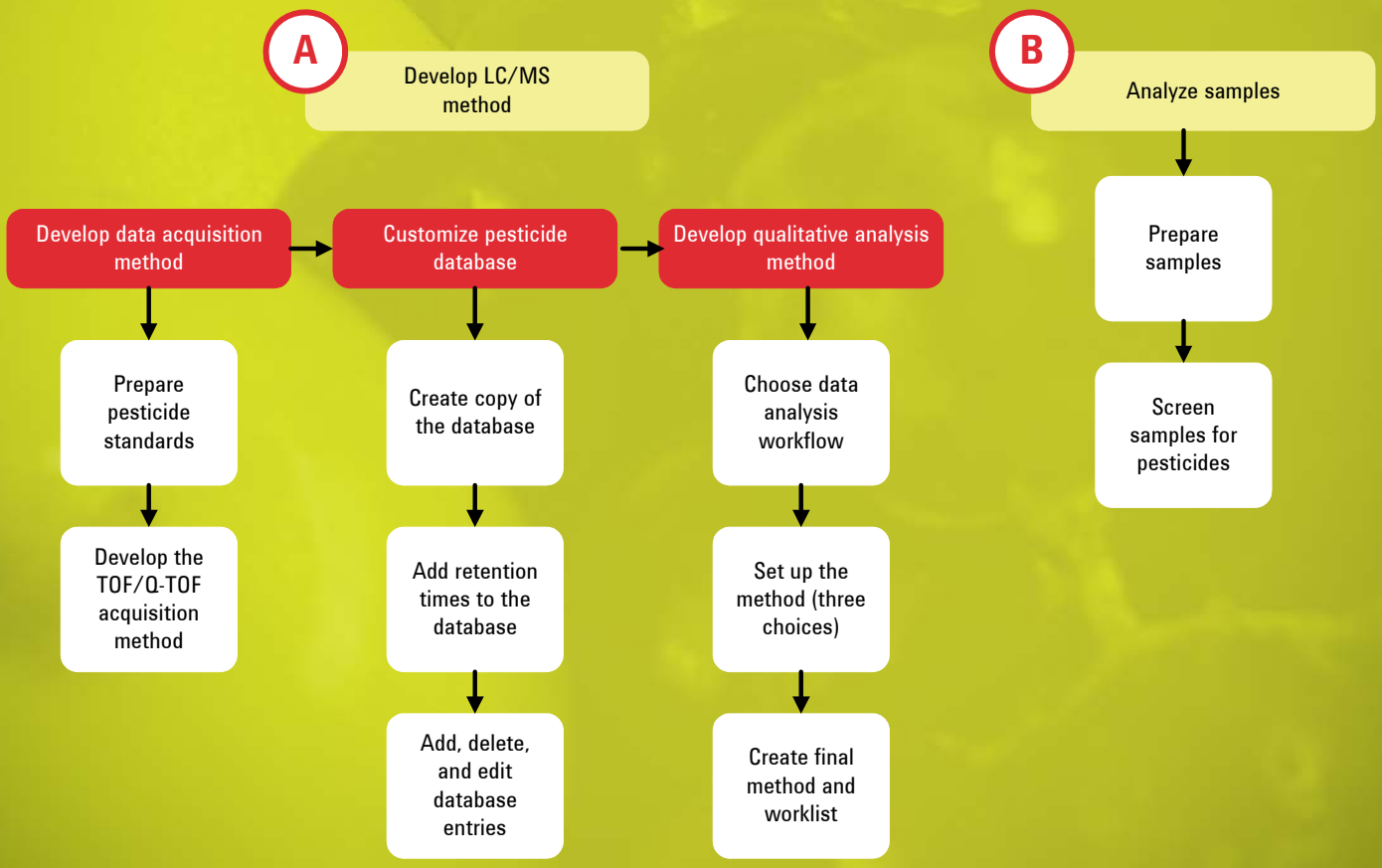
Agilent TOF LC/MS and Q-TOF LC/MS Screening of Pesticides

Workflow Guide



Agilent Technologies

Notices

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Safety Notices

CAUTION

A **CAUTION** notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in damage to the product or loss of important data. Do not proceed beyond a **CAUTION** notice until the indicated conditions are fully understood and met.

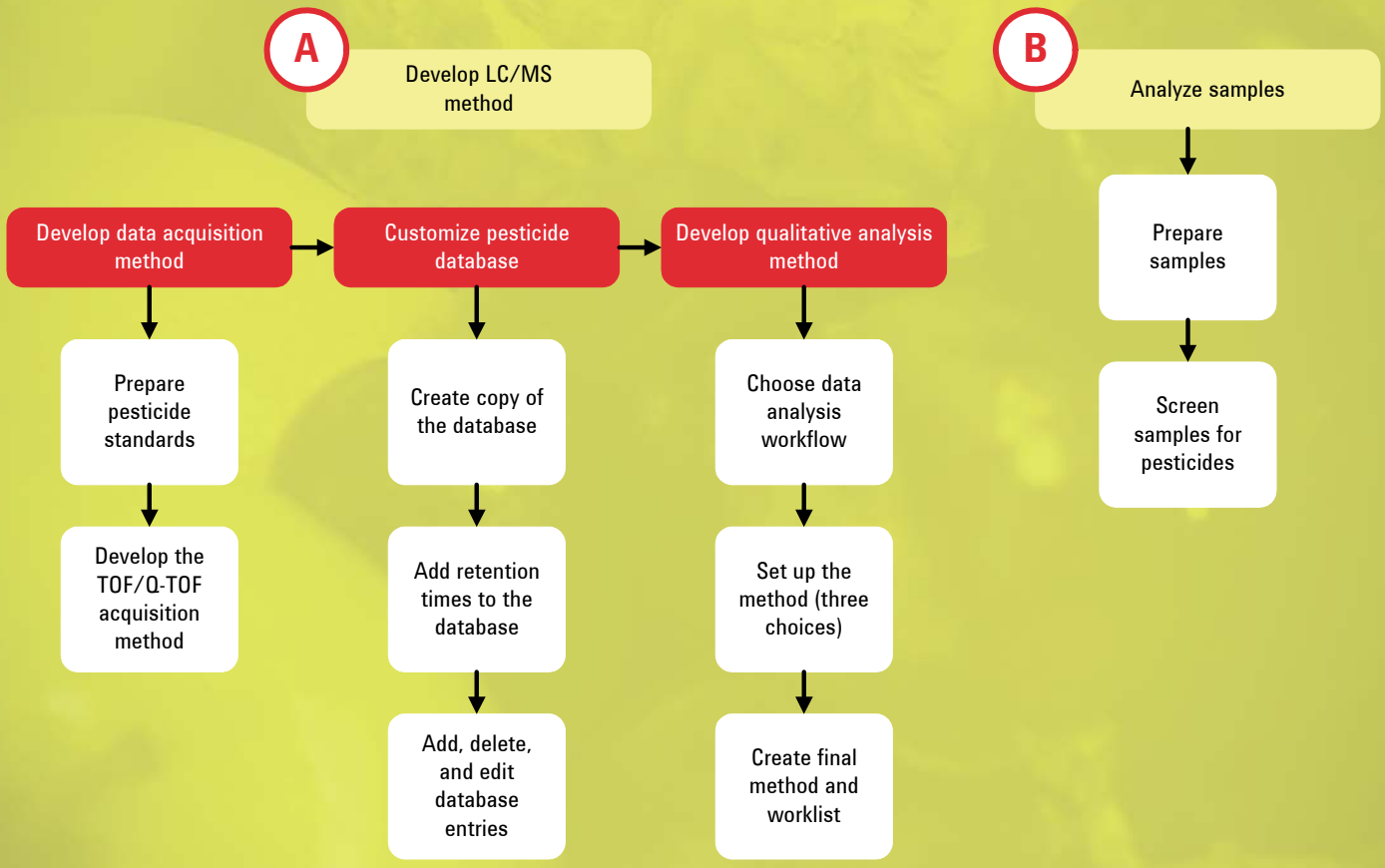
WARNING

A WARNING notice denotes a hazard. It calls attention to an operating procedure, practice, or the like that, if not correctly performed or adhered to, could result in personal injury or death. Do not proceed beyond a WARNING notice until the indicated conditions are fully understood and met.

Contents

Before You Begin

Make sure you read and understand the information in this chapter and have the necessary instrumentation, software, solvents, and lab supplies before you start the analysis.



Introduction

More than 1000 pesticides have been used over the past 100 years, and many have contaminated the environment and our food supply. To protect human health, robust methods are needed to rapidly screen samples for a large number of pesticides, at sensitivity levels that are accepted worldwide.

LC/MS with a time-of-flight (TOF) instrument is ideal for screening, because the TOF (or quadrupole-TOF) delivers full spectra, which enables the analyses of both pesticides that you specifically target, and those that appear unexpectedly.

Accurate-mass pesticide database

To accelerate pesticide analysis with its TOF and Q-TOF instruments, Agilent offers the MassHunter Personal Pesticide Database, which contains exact monoisotopic masses for more than 1600 pesticides and related compounds. The database contains pesticide names, molecular formulas, structures, CAS registry numbers, and links to useful resources. You can easily and semi-automatically add retention times to the database.

Automation

To enable high sample throughput, software tools are needed to automatically and rapidly analyze data. The Agilent MassHunter Qualitative Analysis software has sophisticated compound-finding algorithms that can find all the compounds in a data file. This software can also search those compounds against the MassHunter Personal Pesticide Database to find which of the compounds are pesticides.

Worklists enable complete automation of the analysis, from data acquisition through data analysis and reporting.

Targeted and nontargeted pesticides

This *Workflow Guide* describes how to use the Agilent TOF and Q-TOF LC/MS systems in combination with Agilent software, to perform automated screening for pesticides in food and environmental samples. It describes two types of screening:

- *Targeted screening*, where you look for a specific list of pesticides. You analyze standards under defined conditions and identify pesticides by a database search that uses a combination of accurate mass, isotope match, and retention time.
- *Nontargeted screening*, where you discover pesticides that were not on a specific list. You do not analyze standards for these pesticides, but they are tentatively identified by accurate mass and isotope match from the database search.

NOTE If you plan to do nontargeted screening, it is important to analyze some compounds of known retention time, to make sure the chromatography is stable.

More information

If you need a general introduction to the Agilent TOF or Q-TOF before you begin, see the *Agilent 6200 Series TOF and 6500 Series Q-TOF LC/MS System Concepts Guide*.

You may also view online videos that describe how the 6200 Series TOF works and how the 6500 Series Q-TOF works.

NOTE

This manual gives links to many references. If you have an electronic copy of this manual, you can easily download the documents from the Agilent literature library. Look for and click the blue hypertext; for example, you can click the library link in the previous sentence.

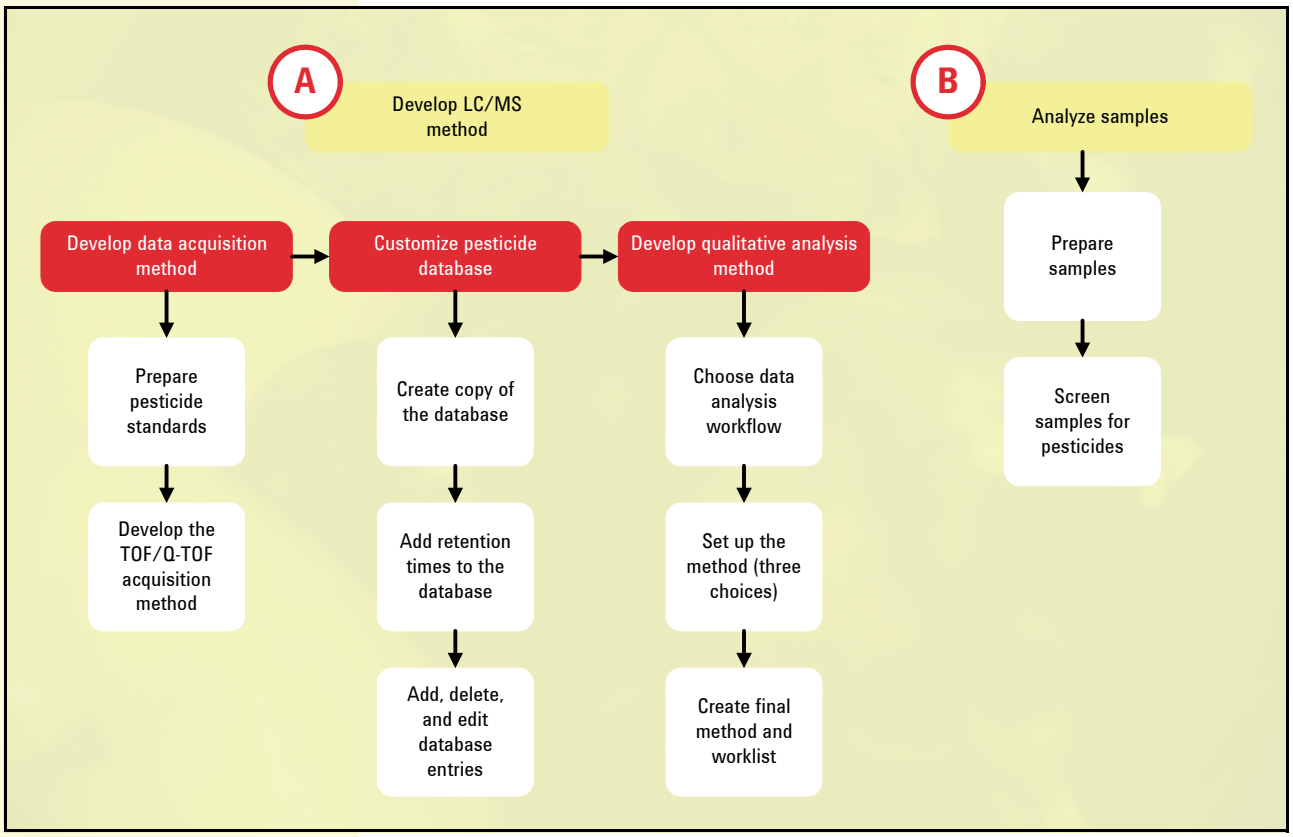
If you have a printed copy, go to the Agilent literature library at **www.agilent.com/chem/library** and type the publication number in the **Keywords** box. Then click **Search**.

Chapter 6 contains a complete list of references.

Overview of the workflow

This manual describes the workflow to do automated multi-residue pesticide screening with the Agilent MassHunter Workstation software and one of these instruments:

- Agilent 6200 Series Accurate-Mass TOF LC/MS System
- Agilent 6500 Series Accurate-Mass Q-TOF LC/MS System



The workflow describes development of the LC/MS method and sample analysis. The workflow uses accurate-mass MS-mode analyses, which you can use to screen for hundreds pesticides with high sensitivity. MS/MS analyses with a Q-TOF provide additional specificity and confirmation, but are not discussed in this *Workflow Guide*.

While you can use this guide to set up an LC/MS analysis of many types of pesticides in many matrices, it is not a compendium of sample preparation methods. It focuses primarily on setting up the LC/MS analysis, doing the data analysis, and automating the entire process.

You can use this workflow as a roadmap for any analysis that requires multi-residue screening on the Agilent TOF or Q-TOF LC/MS Systems. While written specifically for pesticides, most of the concepts apply to other types of analyses.

For a good introduction to pesticide screening with TOF/Q-TOF and an accurate-mass database, see the following:

- "Pesticide Personal Compound Database for Screening and Identification" (Agilent technical note 5990-3976EN, May 2009)

- "An Application Kit for Multi-Residue Screening of Pesticides using LC/TOF or Q-TOF with a Pesticide Personal Compound Database" (Agilent application note 5990-4251EN, August 2009)

Advantages of this workflow

If you need to screen samples for pesticides, this workflow has many advantages:

- Rapid, automated screening of hundreds of pesticides, both targeted and nontargeted
- Ability to do retrospective analyses of the full-spectrum data, to discover other compounds of interest weeks or even years later
- Sensitivity levels in the low ppb range
- Robust analysis
- Ability to customize a copy of the Agilent Personal Pesticide Database, including addition of retention times

What you cannot do with the workflow

This workflow is an excellent way to screen for pesticides, but if a pesticide is not detected, you cannot be absolutely sure that it is not present. This is because the pesticide:

- May not ionize well in electrospray LC/MS
- May not be well-recovered from the sample matrix

To be sure that a given pesticide is *not* present above the level of concern, you must conduct validation studies with the given pesticide, sample matrix, extraction technique, and LC/MS method.

Likewise, even if a pesticide is detected by a combination of accurate mass and retention time, it is not confirmed. For added assurance, you can perform an MS/MS analysis with a Q-TOF instrument and then compare the structure of the pesticide with the fragments you see. The following publication gives more information about this analysis:

- "Q-TOF LC/MS Screening and Confirming of Non-Targeted Pesticides in a Strawberry Extract" (Agilent application note 5990-3935EN, May 2009)

Safety notes

WARNING **Always take proper precautions when you use and dispose of solvents, pesticides, and other chemicals. Read the material data safety sheets supplied by the vendors.**

WARNING **When you disconnect LC columns or fittings, solvents may leak. Use appropriate safety procedures (for example, goggles, safety gloves and protective clothing), especially when you use toxic or hazardous solvents. Read the material data safety sheets supplied by the solvent vendors.**

WARNING **Read, understand, and meet conditions of all warnings in the *Maintenance Guide* that you received with your TOF or Q-TOF instrument. For a list of relevant guides, see the "Manuals" section on page 83 in the Reference chapter.**

CAUTION Read, understand, and meet conditions of all cautions in the *Maintenance Guide* that you received with your TOF or Q-TOF instrument. For a list of relevant guides, see the "Manuals" section on page 83 in the Reference chapter.

Required items

Required hardware and software

Figure 1 *The workflow requires an Agilent LC and TOF or Q-TOF LC/MS System.*

To do this workflow, you need:

- One of the following LCs:
  - Agilent 1220 Infinity LC
  - Agilent 1260 Infinity LC
  - Agilent 1290 Infinity LC
  - Agilent 1200 Series LC system
  - Agilent 1200 Series Rapid Resolution LC system
- One of the following mass spectrometers:
  - Agilent 6200 Series Accurate-Mass TOF LC/MS System
  - Agilent 6500 Series Accurate-Mass Q-TOF LC/MS System
- Agilent MassHunter software:
  - Agilent MassHunter Data Acquisition software for TOF/Q-TOF version B.02.01
  - Agilent MassHunter Qualitative Analysis software version B.04.00 or B.05.00
  - Agilent MassHunter Personal Pesticide Database version B.04.00
  - Agilent MassHunter Personal Compound Database and Library (PCDL) Manager software version B.04.00

The exercises in the next two chapters assume that:

- All instruments have already been installed and are working to specifications.
- You have some experience with pesticide analysis.
- You have been trained on the LC, TOF/Q-TOF instrumentation, and the MassHunter Workstation software. For example, you have taken an operator course at an Agilent training center or you have been trained on-site by an Agilent instructor (Application Engineer or consultant).

Optional kit

The Agilent MassHunter Personal Pesticide Database Kit (G6854AA) is very helpful for this analysis. To save method development time, the kit contains:

- Agilent MassHunter Personal Pesticide Database – required for the workflow (as described above)
- Agilent Personal Compound Database and Library (PCDL) Manager software – required for the workflow
- Positive and negative ion pesticide test mixes
- Agilent ZORBAX Eclipse Plus C18 column, 2.1 mm × 100 mm, 1.8 µm particles
- Trial Agilent SampliQ QuEChERS sample preparation kit
- *Quick Start Guide* that shows you how to analyze the test mixes and create screening methods
- Application and technical notes
- Support disk with examples of easy-to-use screening methods, data files, and reports that demonstrate method setup and adaptation

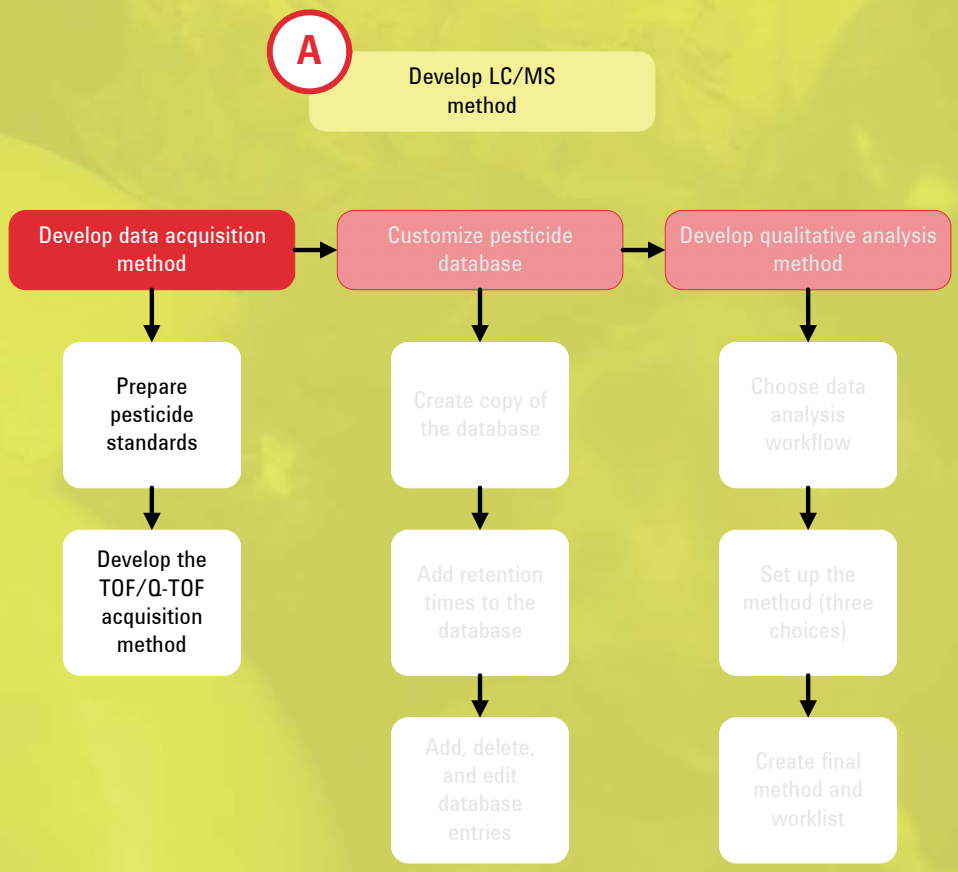
For more information, read "An Application Kit for Multi-Residue Screening of Pesticides using LC/TOF or Q-TOF with a Pesticide Personal Compound Database" (Agilent application note 5990-4251EN, August 2009).

Additional items

See "Required supplies and chemicals" on page 64.

Developing the LC/MS Data Acquisition Method

These exercises show you how to set up a data acquisition method for multi-residue pesticide screening with an Agilent 6200 Series Accurate-Mass TOF LC/MS System (or 6500 Series Accurate-Mass Q-TOF LC/MS System) and MassHunter Workstation software.



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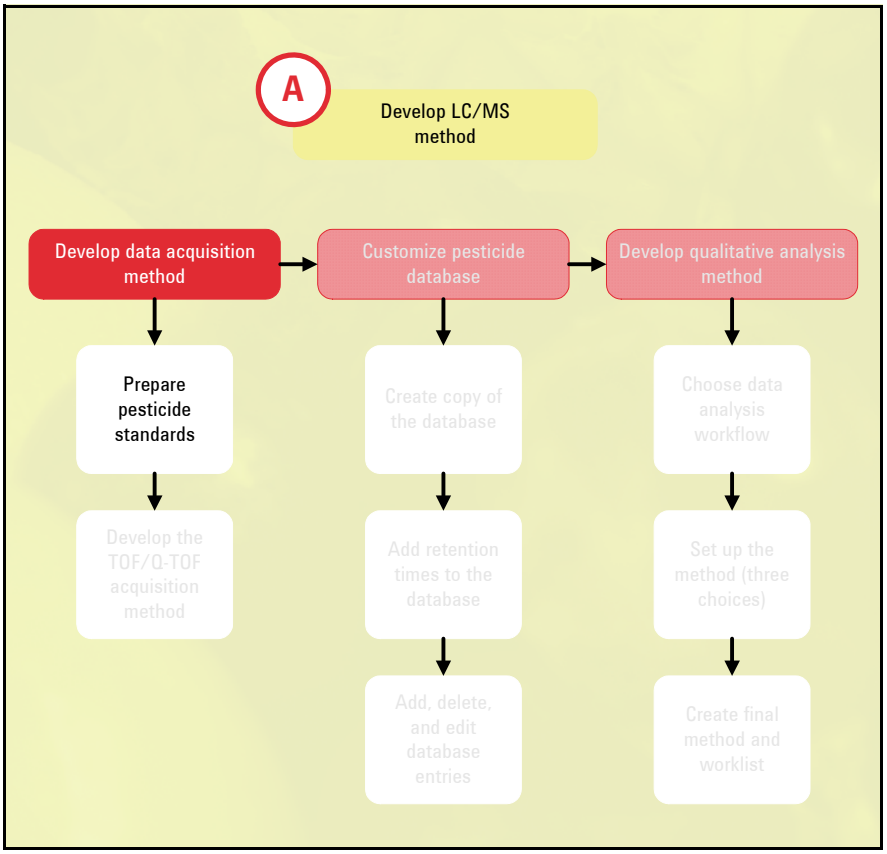
Prepare pesticide standards

In this exercise, you choose an initial LC/MS method. Then you prepare all the pesticide standards you need for method development and sample analysis.



Do preliminary work

1. Choose an initial LC/MS method for data acquisition.

- Do one of the following:
  - Choose one of the methods from the appendix of Agilent application note 5990-4251EN, "An Application Kit for Multi-Residue Screening of Pesticides using LC/TOF or Q-TOF with a Pesticide Personal Compound Database." For your convenience, these settings are also listed in this guide, under "Example LC/MS methods" on page 66.
  - Choose an LC/MS method from another source, or create one yourself. Agilent recommends you still use the ion source settings from Appendix I of 5990-4251EN.

The screening method is MS-only.

The LC method may affect your choice of solvents for the standards.

The *MassHunter Personal Pesticide Database Kit Support Disk* contains example MassHunter method files used to analyze the positive and negative test mixes that

you receive with the kit. These settings are also listed in this guide, under "Example LC/MS methods" on page 66.

2. Purchase any necessary standards and solvents, if you have not already done so.

a Read the all of the steps in the next task to determine which standards you need to purchase.

b Purchase the standards and solvents.

Prepare the standards

1. Prepare stock standards of individual pesticides.

2. Prepare a test mix of selected pesticides.

You will use the test mix to verify that the LC/MS data acquisition method works properly and that parameters in MassHunter Qualitative Analysis are correct for finding compounds.

a Choose a mix of about 8 to 12 pesticides of interest that range in:
- Water solubility (to ensure elution from the LC)
- Ease of ionization and detection by MS (to ensure suitable MS conditions)
- Molecular weight

b Prepare the pesticide mixture such that the concentration of each pesticide is about 100 ng/mL (100 pg/µL) in acetonitrile.
- You will inject 1 to 5 µL.

c You will use this test mixture for "Acquire data for test mixture of pesticides" on page 19.

If you have the Agilent MassHunter Personal Pesticide Database Kit (G6854AA), you may use the positive and negative ion test mixes from that kit. For details, see the *Agilent G6854AA MassHunter Personal Pesticide Database Kit Quick Start Guide* (Agilent publication 5990-4262EN, August 2009).

If you prepare your own test mix, some pesticides may need higher or lower concentrations. The concentration should give a good MS response, but not be so high that you see carry-over in blanks.

3. (Optional) Prepare retention time standard(s).

You will use the standard(s) to determine retention times for targeted analysis, or to determine retention times of selected pesticides for quality assurance purposes.

a Note any isobaric or nearly isobaric pesticides, and prepare them in separate mixtures.

b Prepare pesticide mixture(s) such that the concentration of each pesticide is about 100 ng/mL (100 pg/µL) in acetonitrile.
- You will inject 1 to 5 µL.

c You will use these standards for "Analyze pesticides to get retention times" on page 27.

The test mix you prepared in step 2 can also be a retention time standard, so you do not need to prepare another standard for those pesticides.

Some pesticides may need higher or lower concentrations. The concentration should give you a good response, but not be so high that you see carry-over.

4. Prepare additional standards required to ensure that your method works properly.

Additional standards may include:
- Standards used to spike samples to determine recoveries
- Matrix-matched standards
- Internal standards added to sample extracts prior to analysis

You will use these standards when you analyze samples (Chapter 5).

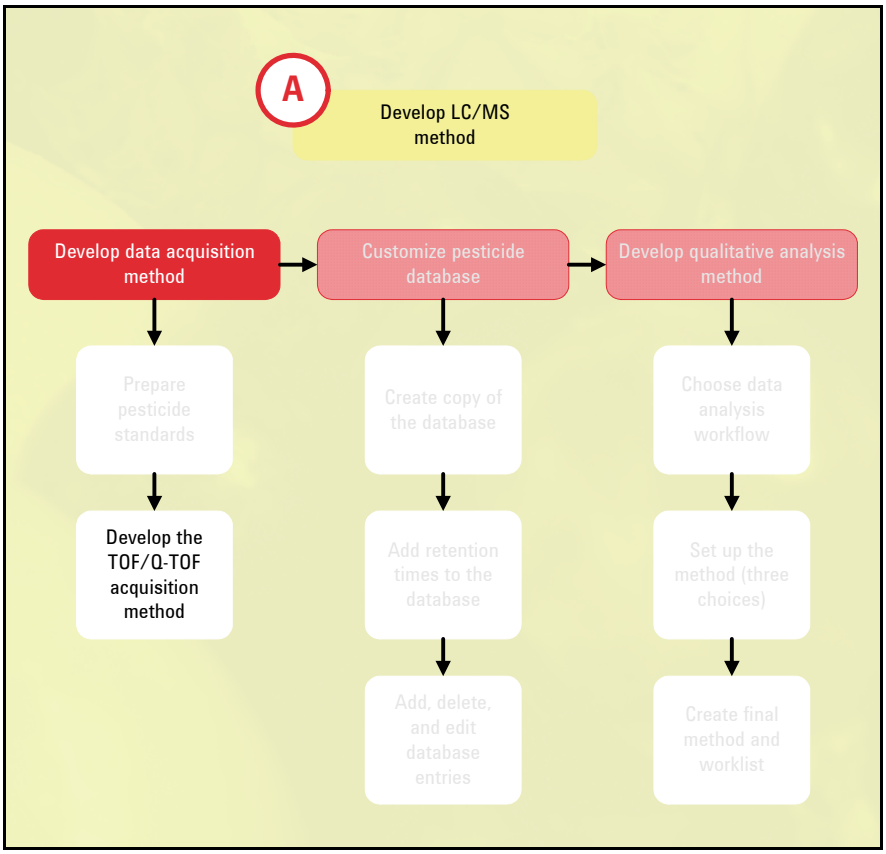
Matrix-matched standards are important for understanding the influence of matrix contaminants on identification of unknowns.

Some pesticides precipitate if diluted in solvent that contains a high percentage of water. In these cases, Agilent recommends that you use an autosampler program to dilute the sample before injection onto the column. For example, you may use a stacked ("sandwich") injection to successively draw aliquots of water, nonaqueous standard, and more water.

5. Refrigerate the standards.

Develop the TOF/Q-TOF data acquisition method

In this exercise, you develop the data acquisition method for the 6200 Series Accurate-Mass TOF or 6500 Series Accurate-Mass Q-TOF LC/MS System. For screening, you will use an MS-only method. An MS/MS method is excellent for confirmation, but is not discussed here.



Prepare the LC/MS system

1. Prepare LC solvents.

   a Prepare the aqueous and organic mobile phases for the method you chose in "Prepare pesticide standards" on page 14.

   b Put the solvent bottles on the LC.

2. Start the MassHunter Data Acquisition program.

   • Double-click the MassHunter Data Acquisition icon.

If you need help, see Step 1 in the "Getting Started" section of the *Agilent 6200 Series TOF and 6500 Series Q-TOF LC/MS System Quick Start Guide*.

3. Prepare the LC modules.

   a Switch the LC stream to waste (or disconnect it from the MS).

   b Purge the LC pump.

   c Install the column and condition it as described in the column instructions included in the column package.

   d Set up to view real-time parameter values (actuals).

   e Set up to display real-time plots.

   If you need help, see Step 2 in the "Getting Started" section of the *Agilent 6200 Series TOF and 6500 Series Q-TOF LC/MS System Quick Start Guide*.

   It is very important to purge solvent channels A and B because trapped air causes irreproducible retention times for analytes.

4. Prepare the Agilent TOF or Q-TOF.

   a Autotune or calibrate the MS as needed.

   b Switch the LC stream to MS.

   c Start the flow at initial method conditions.

   d Monitor the MS baseline and spectral displays.

   If you need help, see Step 3 in the "Getting Started" section of the *Agilent 6200 Series TOF and 6500 Series Q-TOF LC/MS System Quick Start Guide*.

Set up LC/MS parameters

This task gives you the basic steps to set up an LC/MS method with the MassHunter Data Acquisition program.

If you have the Agilent MassHunter Personal Pesticide Database Kit (G6854AA), you may start with the methods from that kit, which will save time. For details, see the *Agilent G6854AA MassHunter Personal Pesticide Database Kit Quick Start Guide* (Agilent publication 5990-4262EN, August 2009).

1. Enter values for all the LC modules.

   - In the MassHunter Data Acquisition program, click each LC tab and enter values from the method you chose in step 1 on page 14.

   If you need help with setting up a method, see:
   - Step 4 in the "Getting Started" section of the *Agilent 6200 Series TOF and 6500 Series Q-TOF LC/MS System Quick Start Guide*.
   - Chapter 1, Task 2 of the *Agilent MassHunter Workstation Software – Data Acquisition for 6200 Series TOF and 6500 Series Q-TOF Familiarization Guide* (Agilent publication G3335-90066, Third Edition, May 2009).

2. Enter TOF or Q-TOF MS parameters.

   a Click the **MS TOF** or **MS Q-TOF** tab and enter values from the method you chose in step 1 on page 14.

   b For MS conditions, use the settings in Appendix I of Agilent application note 5990-4251EN as a starting point. These settings are also listed in this guide, under "Example LC/MS methods" on page 66.

   c Click the **General** tab. Under Data Storage, click **Both**. That way, you acquire both centroid and profile spectra.

   When you later analyze the data, the Find by Formula algorithm can use centroid data for extracted ion chromatograms (EICs), which makes EIC generation much faster.

3. Save the method.

   a Click **File > Save As > Method**.

   b Give the method a name and click **OK**.

Acquire data for test mixture of pesticides

In this task, you make sure that the LC/MS method that you created in the last task works properly. To do that, you analyze the pesticide test mix (100 pg/µL per pesticide) that you prepared in step 2 on page 15.

If you have the Agilent MassHunter Personal Pesticide Database Kit (G6854AA), you may use the positive and negative ion test mixes from that kit. For details, see the *Agilent G6854AA MassHunter Personal Pesticide Database Kit Quick Start Guide* (Agilent publication 5990-4262EN, August 2009).

1. Open MassHunter Data Acquisition, if it is not already open.

2. Put the test mix standard into the LC autosampler.

3. Load your method.

   a Click **File > Open > Method**.

   b Select the method you created in "Set up LC/MS parameters" on page 18, then click **OK**.

4. Set up to analyze the test mix.

   a In the Method pane, click the **Sample** tab.

   b Under **Sample**, type the name and position.

   c Under **Data File**, type the **Name** and enter the **Path**.

   d Enter the **Run** parameters.

   e Click **Apply**.

If you need practice to analyze a sample, see the following:

- Step 4 in the "Getting Started" section of the *Agilent 6200 Series TOF and 6500 Series Q-TOF LC/MS System Quick Start Guide*.
- Task 1 in the "Set up and run single samples and worklists" section of the *Agilent MassHunter Workstation Software – Data Acquisition for 6200 Series TOF and 6500 Series Q-TOF Familiarization Guide* (Agilent publication G3335-90066, Third Edition, May 2009).

5. Start the run.

a Click the **Single Sample** button on the toolbar.

b Wait for the run to finish.

c Verify that your analysis was successful.

You can run the single sample in either locked or unlocked mode. To change, click the **Lock** button on the toolbar.

When the mode is locked, no one can change the method or the worklist while the single sample or worklist is running.

View results and adjust method, if necessary

In this task, you use MassHunter Qualitative Analysis to find the compounds in the data file that you created in the last task. Then you look at the results to see if all the pesticides in the standard appeared in the data file.

If you have the Agilent MassHunter Personal Pesticide Database Kit, see the *Agilent G6854AA MassHunter Personal Pesticide Database Kit Quick Start Guide* (Agilent publication 5990-4262EN, August 2009) for the correct procedure to use with the test mixes in that kit.

1. Open MassHunter Qualitative Analysis.

- Double-click the MassHunter Qualitative Analysis icon.

2. Open the data file you created in the last task.

a Click **File > Open Data File**.

b Select the data file and click **Open**.

3. Use the MS Target Compound Screening workflow.

a Click **View > Configure for Workflow > MS Target Compound Screening**.

b If you see a message that describes what the program will do next, click **OK**.

The Method Explorer changes with each workflow. If you have difficulty with the instructions in this manual, check that you are using the correct workflow.

4. Find compounds by formula.

a In Method Explorer, click **Find Compounds by Formula > Find by Formula – Options**.

b For Source of formulas to confirm, click **These formulas**.

c Type or copy the formulas into the box. Separate them with commas.

It may be easier to assemble all the formulas in a text file, then copy them into the box.

You can get the formulas from the Agilent MassHunter Personal Pesticide Database.

- Open the MassHunter Personal Compound Database and Library (PCDL) Manager software.
- In the **Single Search** tab, type the pesticide name in the **Name** box.
- Click the **Find Compounds** button on the toolbar.

d Depending on the polarity of your analysis, click the **Positive Ions** tab or the **Negative Ions** tab.

e Mark check boxes for the expected adduct ions.

f For additional settings and tips, see "Initial settings for Find by Formula" on page 75.

g Click the button to run the Find Compounds by Formula algorithm on the data file.

5. Display the results to see if all the pesticides were found.

6. If some pesticides were not found, change the Find by Formula settings, if necessary.

a Compare your Find by Formula settings with those in "Initial settings for Find by Formula" on page 75.

b If necessary, change the settings and run Find Compounds by Formula again.

7. If some pesticides were not found, and the Find by Formula settings are OK, use extracted ion chromatograms (EICs) to search for the pesticides.

a Calculate the correct mass of a missing pesticide (**Tools > Show Mass Calculator**).

b Generate an EIC of the pesticide (**Chromatograms > Extract Chromatograms**).

- On the MS Chromatogram tab, for **Type**, select **EIC** and type the **m/z value**.
- On the Advanced tab, set **Single m/z expansion for this chromatogram** to **Symmetric (ppm)**, with a value of **50**.

c From the EIC, obtain a background-subtracted, averaged spectrum.

d Search the database for the masses in the spectrum.

e Repeat as necessary for additional pesticides.

8. If some pesticides are still missing, run the analysis again.

- Inject more sample or prepare and analyze a standard at higher concentration.

Injection of 1 ng on-column should give sufficient signal for Find by Formula.

9. If some pesticides still were not found, determine why.

a Determine why the pesticides were not detected. Some examples are:
   - Run time too short
   - Pesticide degraded
   - Solution chemistry not right for ion formation
   - Pesticide does not ionize in electrospray MS
   - Pesticide ionizes in opposite polarity

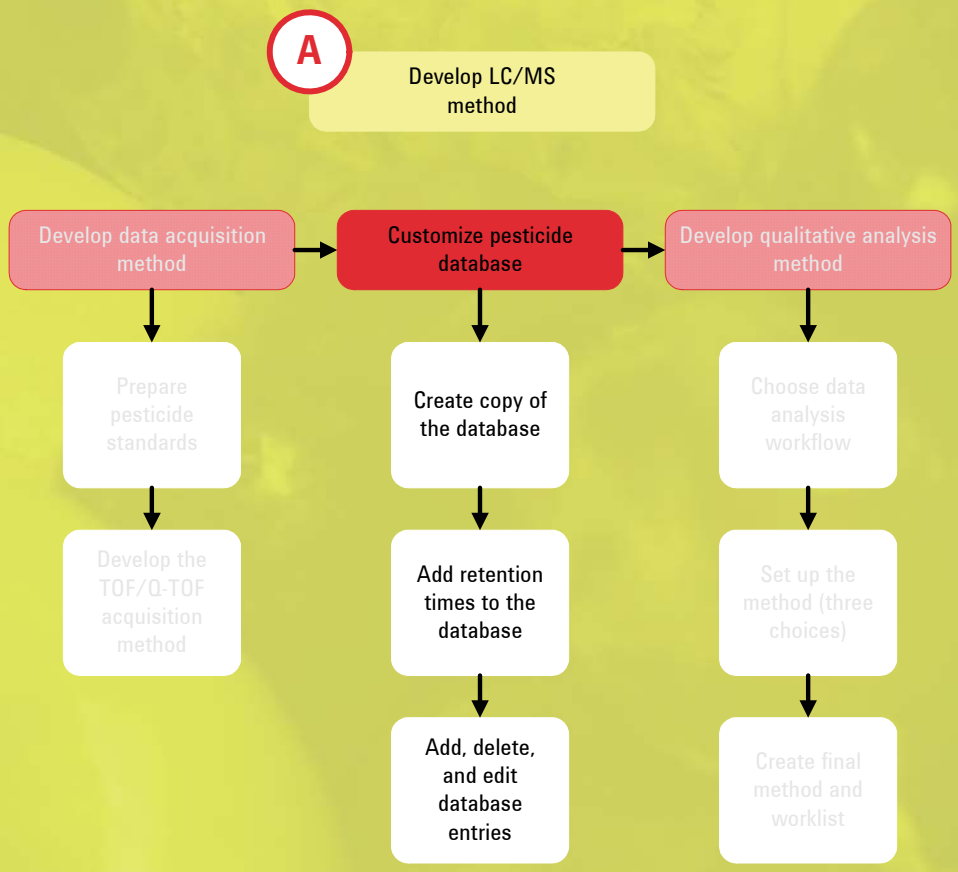
b Make any necessary changes.

c Run the analysis again to verify that the pesticides were detected.

d Save the final method.

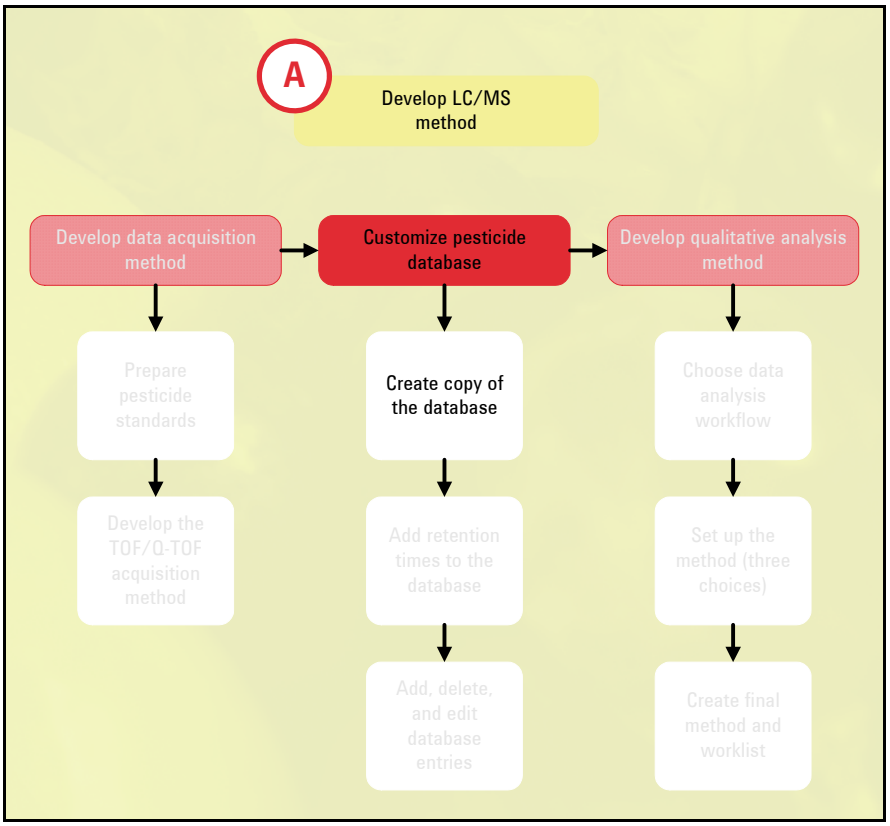
Customizing the Personal Pesticide Database

These exercises show you how to set up a custom pesticide database that contains the retention times of pesticides of interest. They also describe how to add new pesticides to the custom database, how to edit the database records, and how to delete database entries.



Create a copy of the database

The Agilent MassHunter Personal Pesticide Database is "read-only," so you cannot change the original. In this exercise, you create a copy of the Agilent MassHunter Personal Pesticide Database so that you can customize it.



For a good introduction to the Agilent MassHunter Personal Pesticide Database, see the following:

- "Pesticide Personal Compound Database for Screening and Identification" (Agilent technical note 5990-3976EN, May 2009)

1. Open the MassHunter Personal Compound Database and Library (PCDL) Manager software.

a Double-click the PCDL Manager icon on your desktop.

b In the Open Database/Library dialog box, navigate to the **MassHunter\PCDL** folder.

c Click **Pesticides.cdb**, then click **Open**.

If you need help, see "Startup" in the "Installation" section in the *Agilent G6854 MassHunter Personal Pesticide Database Quick Start Guide* (Agilent publication G6854-90003, First Edition, July 2009).

2. Create a copy of the Agilent MassHunter Personal Pesticide Database, so you can customize it.

a Click **File > New Database/Library**.

b Browse to **\databases** as the **Database/library path**, since the default path is **\Library**.

c Select **Pesticides** as the starting database.

d Select **Pesticides** as the database type.

e Type a name for the new database.

f Enter a description for the new database.

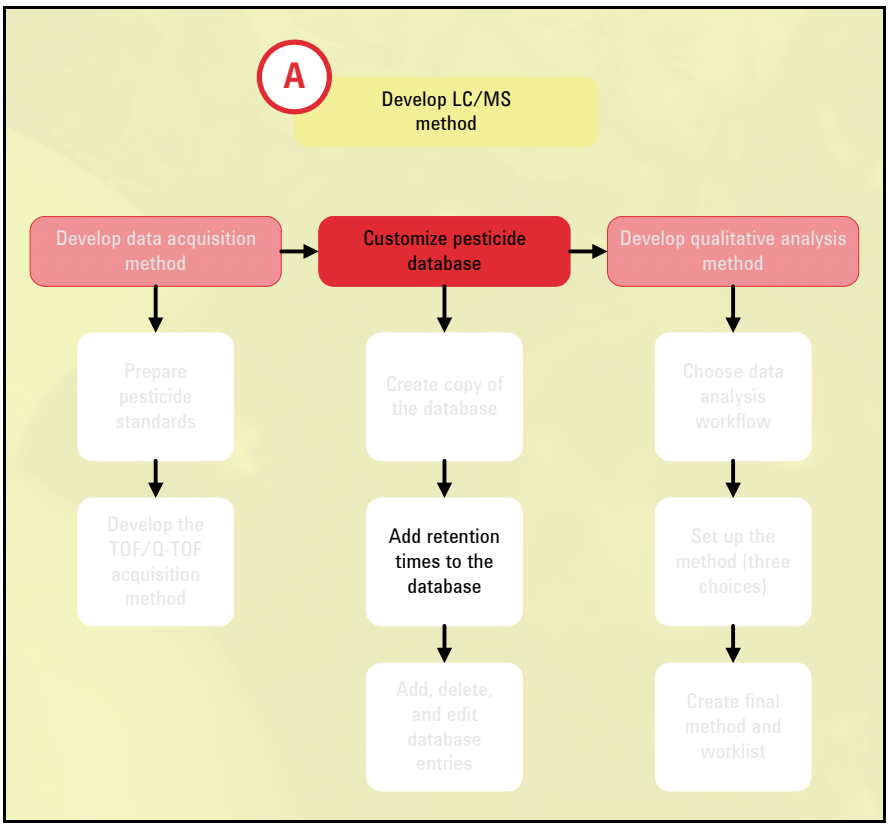
g Click the **Create** button.

If you need help, see the *Agilent G6854 MassHunter Personal Pesticide Database Quick Start Guide* (Agilent publication G6854-90003, First Edition, July 2009). In the "Familiarization Exercises" section, see "Exercise 3. Create a custom database."

Add retention times to the database

In this exercise, you analyze retention time standards and then use MassHunter Qualitative Analysis to find the pesticides in the data files. You then export the masses and retention times so you can later import them into a custom pesticide database. The automation features in MassHunter Qualitative Analysis and the MassHunter Personal Compound Database and Library (PCDL) Manager software allow you to quickly add hundreds of retention times to a custom library.

Even if you plan to do nontargeted analysis, Agilent recommends that you add retention times for some compounds to the Agilent MassHunter Personal Pesticide Database. These retention times allow you to verify that the chromatography is stable and help you to check that the analysis is working properly.



Analyze pesticides to get retention times

1. Open MassHunter Data Acquisition, if it is not already open.
2. Put the retention time standards into the LC autosampler.
3. Set up a worklist to analyze the retention time standards.

You do this task only if you want to add retention times to the database. Retention times allow you to do target analyses for the compounds of interest, and provide a check of chromatography for quality assurance purposes.

- Use the standards that you prepared in step 3 on page 15.

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a If necessary, click the **Worklist** button on the toolbar to display the Worklist pane. 

b To set up the worklist run, click **Worklist > Worklist Run Parameters**.

c Set the **Data File Path**, verify that the other parameters are set properly, and click **OK**.

d Click **Worklist > Add Multiple Samples**.

e Type or select the necessary information in the Sample Information tab. Be sure to:
- Select the method you developed in Chapter 2.
- Type an injection volume of 2.

f Click the **Sample Position** tab.

g Drag to select the sample positions, then click **OK**. (See Figure 2.)

h Verify that the worklist has been populated with samples (Figure 2).

i Edit the worklist table as necessary.

If you need practice to run a worklist, see the following:
- End of step 4 in the "Getting Started" section of the *Agilent 6200 Series TOF and 6500 Series Q-TOF LC/MS System Quick Start Guide*.
- Task 2 in the "Set up and run single samples and worklists" section of the *Agilent MassHunter Workstation Software – Data Acquisition for 6200 Series TOF and 6500 Series Q-TOF Familiarization Guide* (Agilent publication G3335-90066, Third Edition, May 2009).

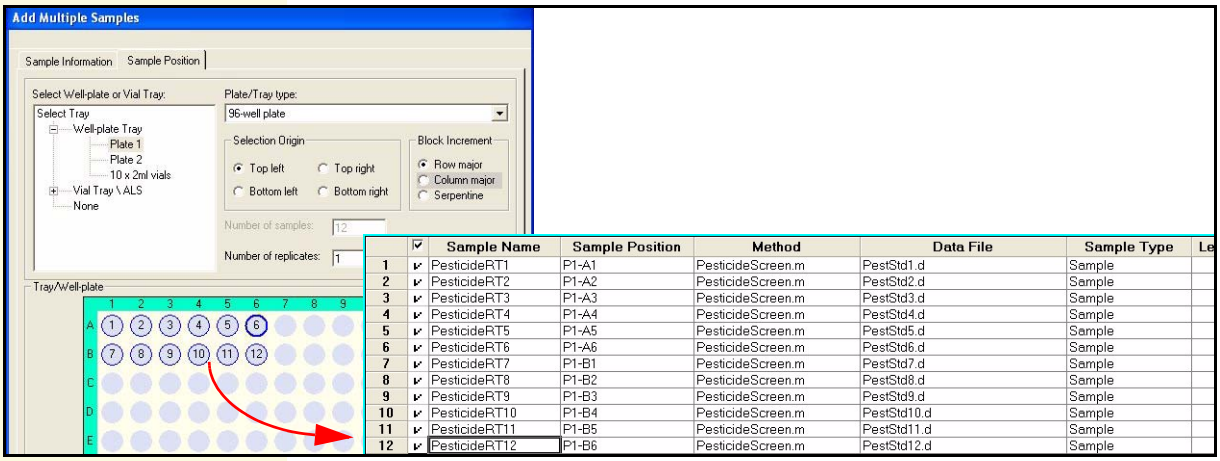


Figure 2 *The worklist is filled in automatically from your sample selection. You can then edit it.*

4. Run the worklist.

a Click the **Run Worklist** button on the toolbar.

b Wait for the worklist to finish.

c Verify that your analysis was successful.

You can run the worklist in either locked or unlocked mode. When the mode is locked, no one can change the method or the worklist while the worklist is running.

Export retention times from data files

This task shows you how to automatically find the compounds in a data file and export the compound information to a CSV file so that you can (in the next task) import the retention times into your custom pesticide database.

1. Open MassHunter Qualitative Analysis.

- Double-click the MassHunter Qualitative Analysis icon.

2. Use the MS Target Compound Screening workflow.

a Click **View > Configure for Workflow > MS Target Compound Screening**.

b If you see a message that describes what the program will do next, click **OK**.

3. Make sure retention times use minutes as units.

a Click **Tools > Plot Display Options**.

b Click the **Chromatogram** tab.

c For **Retention time units**, click **Minutes**.

d Click **OK**.

4. Save the method with a new name.

   a Click **Method > Save As**.

   b Give the method a new name (for example, **Pesticide-RTs-MFE.m**) and click **OK**.

5. Determine the spectral noise level.

   a Click **File > Open Data File**.

   b Select a data file that you created in "Analyze pesticides to get retention times" on page 27 and click **OK**.

   c Display a spectrum.

   d Determine the approximate noise level. You need this number in the next step.

6. Use the molecular feature extractor (MFE) to find all the compounds in the data file.

   a Follow the MFE instructions in the next chapter. See step 5 on page 44 through step 10 on page 45.

   b Click **Method > Save**.

   c Click the button to start **Find by Molecular Feature**.

   d To evaluate the MFE results, review the found compounds and see if you detected too many or too few. You may need to adjust the settings, particularly **Use peaks with height** on the Extraction tab. (See "Extraction tab" on page 69.) Then try again, if necessary.

   If you need practice with MFE, see the *Agilent MassHunter Workstation Software Qualitative Analysis Familiarization Guide*.

   For tips on use of MFE, as well figures that show settings, see "Initial settings for Find by Molecular Feature Extraction" on page 69.

7. Export the mass list to a CSV file.

   a Click **File > Export > as Compound Summary CSV**.

   b In the Export Compound Summary CSV Options dialog box, enter settings as shown in Figure 3.

   c Click **OK**.

   NOTE

   As an alternative, you can copy and paste a mass list into the MassHunter PCDL Manager software rather than importing a CSV file. To copy and paste a mass list:

   a In MassHunter Qualitative Analysis, make sure the Compound List is displayed. (Click **View > Compound List**.)

   b Select the entire Compound List.

   c Right-click in the Compound List and click **Copy to Clipboard**.

   d In MassHunter PCDL Manager software, click the **Batch Search** tab.

   e Click somewhere in the table and click **Paste Mass List**.

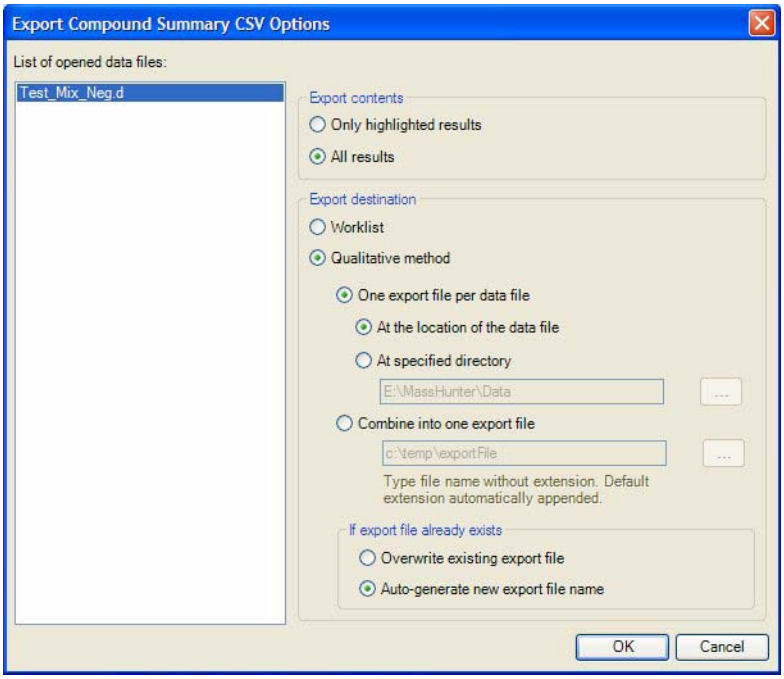


Figure 3 *Settings to export a compound summary CSV file*

To update retention times, the PCDL Manager software needs only the accurate mass and retention time. The CSV file you export in this step contains many more columns than that, but the PCDL Manager software finds the columns it needs and ignores the rest.

8. Repeat step 5 through step 7 for any additional data files that contain the retention times you need.

Update the pesticide database with retention times

In this task, you add pesticide retention times to your custom pesticide database.

1. Open the MassHunter Personal Compound Database and Library (PCDL) Manager software, and your custom pesticide database.

   a Double-click the PCDL Manager icon on your desktop.

   b In the Open Database/Library dialog box, navigate to the **MassHunter\PCDL** folder.

   c Click the name of the custom pesticide database that you created in "Create a copy of the database" on page 24, then click **Open**.

2. Enable editing for this database.

   a Open the **Database/Library** menu.

   b Mark **Allow Editing** if it is not currently marked.

3. Click the **Batch Search** tab.

4. Open a CSV file that you created in the previous task.

   a Click the **File** button to the left of the mass list table.

   b Browse to the correct folder (under **\MassHunter\Data**) and select the file.

   c Click the **Open** button.

   This file contains masses and retention times.

   Alternatively, you can paste masses and retention times into the MassHunter PCDL Manager software.

5. Set the search parameters.

   a Set the **Mass tolerance** to **3 ppm**.

   b Mark **Retention times Optional**.

   c Set the **RT tolerance** to 1 minute.

6. Start the search.

   a Click the **Find Compounds** toolbar button.

   b In the mass list table, click the heading for **Mass**, to sort the table by mass.

   c Observe that the number of hits for each mass appears in the Hits column of the mass list. Note that conflicting hits are shown in red text. See Figure 4.

   You may see two types of conflicting (red) hits:
   - When the Hits column contains a number greater than 1, that means the database contains more than one hit for that mass, so you need to choose the correct hit.
   - When the Hits column contains the number 1, that means that identical or nearly identical mass occurred at different retention times, and both instances matched to the same database hit. You need to choose the correct mass/retention time

pair for the hit. Because you sorted the table by mass, you can easily see the two mass/retention time pairs that matched to the same hit.

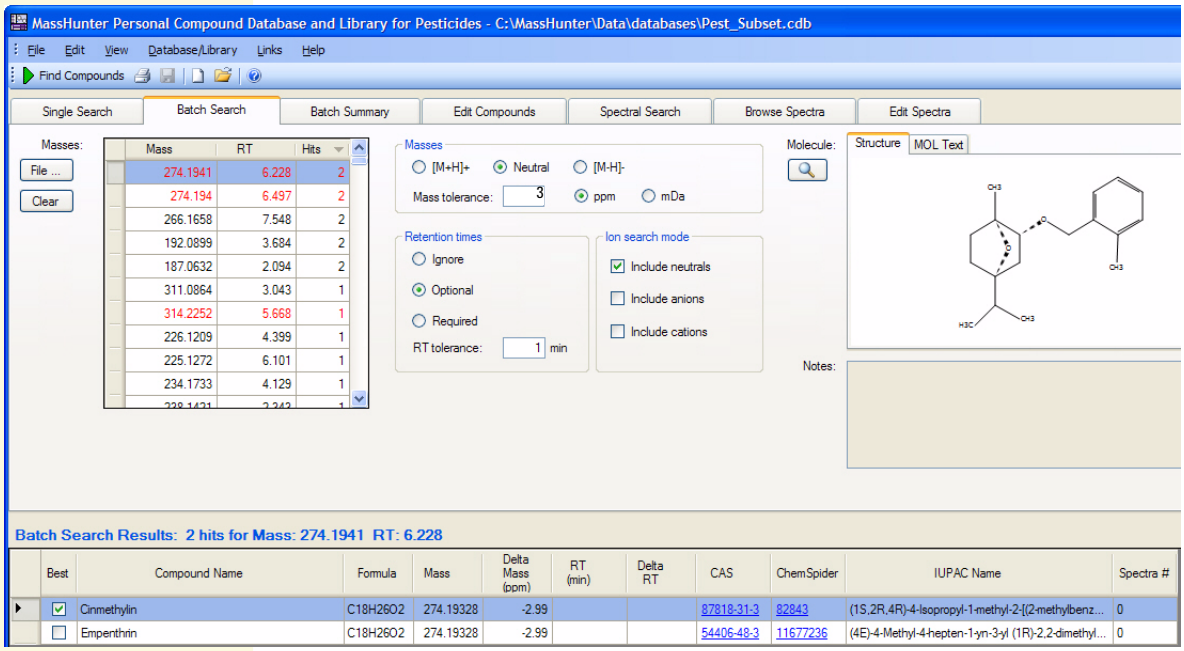


Figure 4 *Batch Search results with retention time optional*

7. View hits for selected masses.

- Click a row in the Mass List table to display the compounds found for that mass. These compounds appear in the Batch Search Results table in the lower part of the window.

For easy review of results, use the **F4** key to scroll down the Mass List and the **Shift**+**F4** keys to scroll back up. The information in the results table is updated as you scroll through the mass list.

8. Resolve any results that conflict (displayed in red).

a If a given mass matched to two database hits, mark the **Best** column for the row (compound) you believe is the best compound hit for that mass. In many cases, this will be the one already selected by the software.

b If two masses at different retention times (two compounds) matched to the same database hit, clear the checkbox for one of the masses. The Delta Mass (ppm) column may provide a clue about the correct result. The retention time may also provide a clue.

9. Click the **Batch Summary** tab.

- See Figure 5.

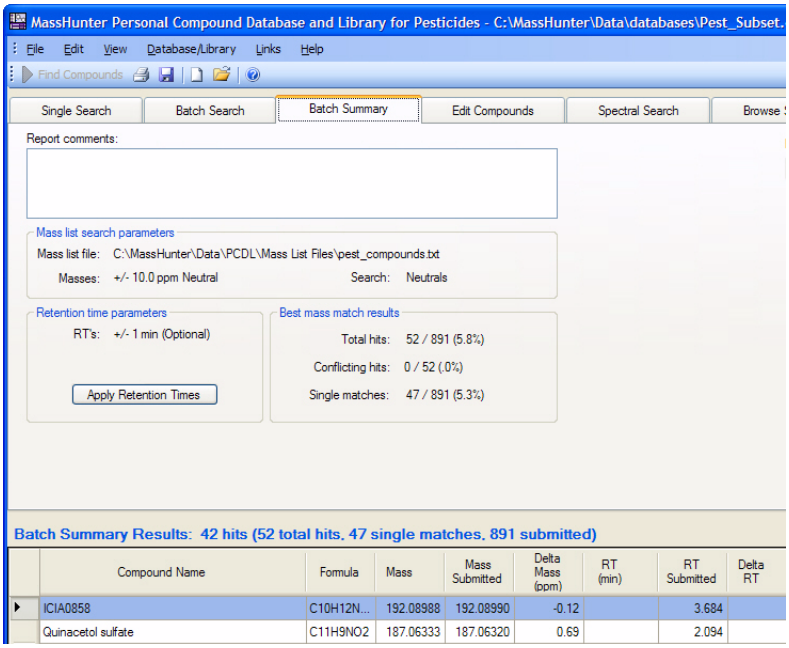


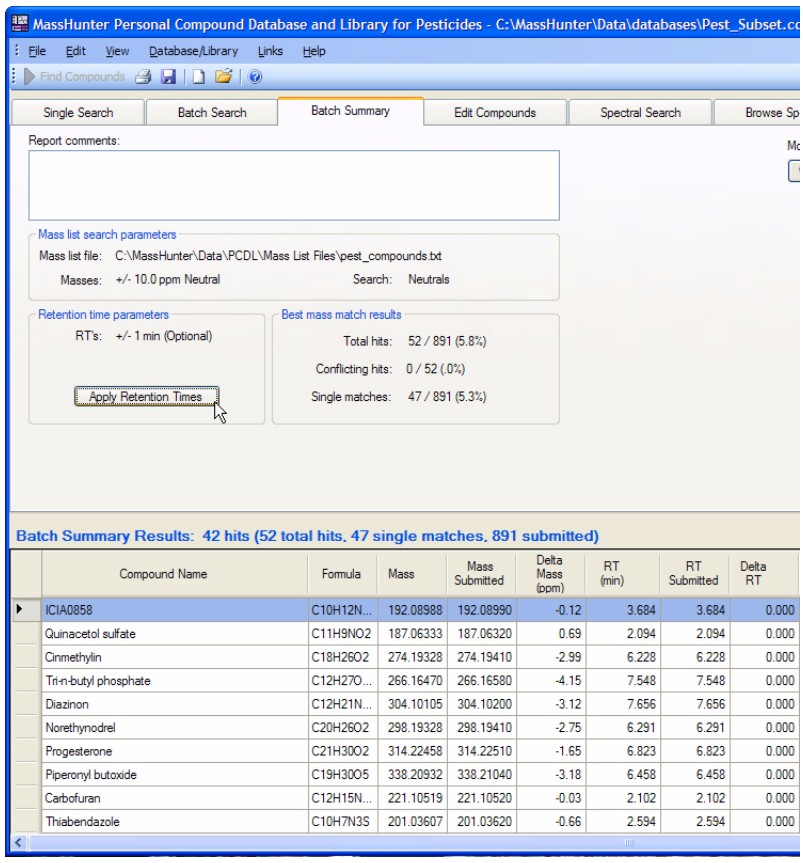
Figure 5 *Batch Summary results with retention time optional (before updating)*

10. Update retention times in the database from the current mass list.

a Click the **Apply Retention Times** button.

b Note that this button is unavailable until the database is enabled for editing (as described in step 2) and all conflicting hits are resolved (as described in step 8).

Retention times are updated in the database for each of the compounds listed in the Batch Summary table. See Figure 6.



Compound Name	Formula	Mass	Mass Submitted	Delta Mass (ppm)	RT (min)	RT Submitted	Delta RT
ICIA0858	C10H12N...	192.08988	192.08990	-0.12	3.684	3.684	0.000
Quinacetol sulfate	C11H9NO2	187.06333	187.06320	0.69	2.094	2.094	0.000
Cinmethylin	C18H26O2	274.19328	274.19410	-2.99	6.228	6.228	0.000
Tri-n-butyl phosphate	C12H27O...	266.16470	266.16580	-4.15	7.548	7.548	0.000
Diazinon	C12H21N...	304.10105	304.10200	-3.12	7.656	7.656	0.000
Norethynodrel	C20H26O2	298.19328	298.19410	-2.75	6.291	6.291	0.000
Progesterone	C21H30O2	314.22458	314.22510	-1.65	6.823	6.823	0.000
Piperonyl butoxide	C19H30O5	338.20932	338.21040	-3.18	6.458	6.458	0.000
Carbofuran	C12H15N...	221.10519	221.10520	-0.03	2.102	2.102	0.000
Thiabendazole	C10H7N3S	201.03607	201.03620	-0.66	2.594	2.594	0.000

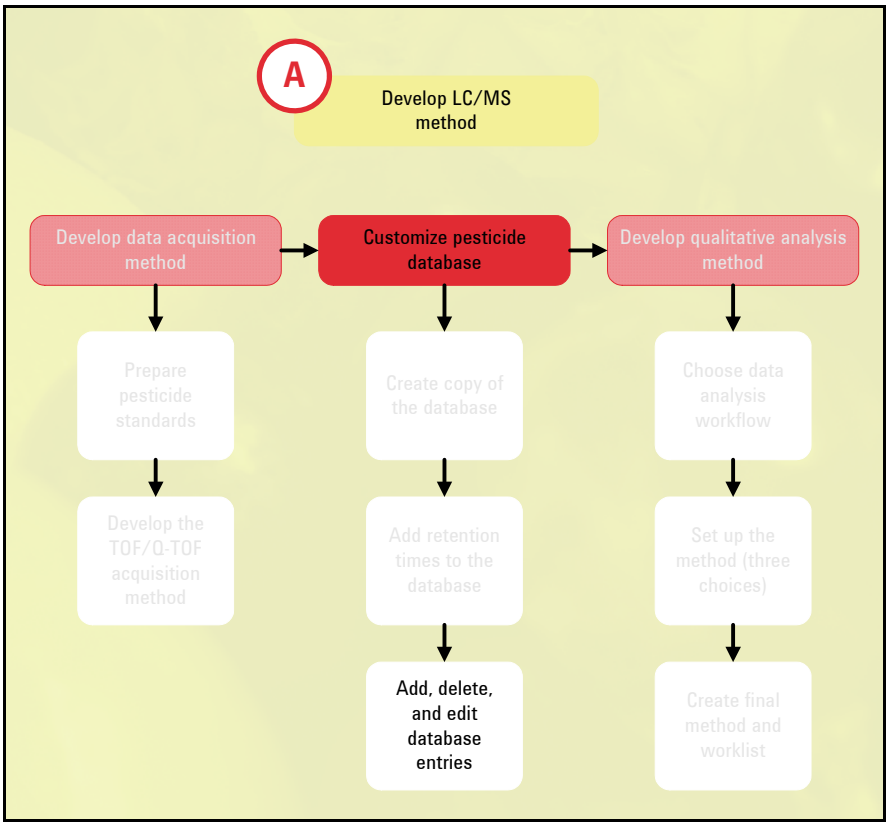
Figure 6 *Batch Summary results with retention time times updated*

The retention times are saved automatically. You do not need to resave the database.

11. Repeat step 3 through step 10 for any additional CSV files that you created.

Add, delete, and edit database entries

This exercise shows you how to add new pesticides to the database, delete existing ones, and edit the information in the database.



1. Open the MassHunter Personal Compound Database and Library (PCDL) Manager software, and your custom pesticide database.

   a Double-click the PCDL Manager icon on your desktop.

   b In the Open Database/Library dialog box, navigate to the **MassHunter\PCDL** folder.

   c Click the name of your custom pesticide database, then click **Open**.

2. Enable editing for this database.

   a Open the **Database/Library** menu.

   b Mark **Allow Editing** if it is not currently marked.

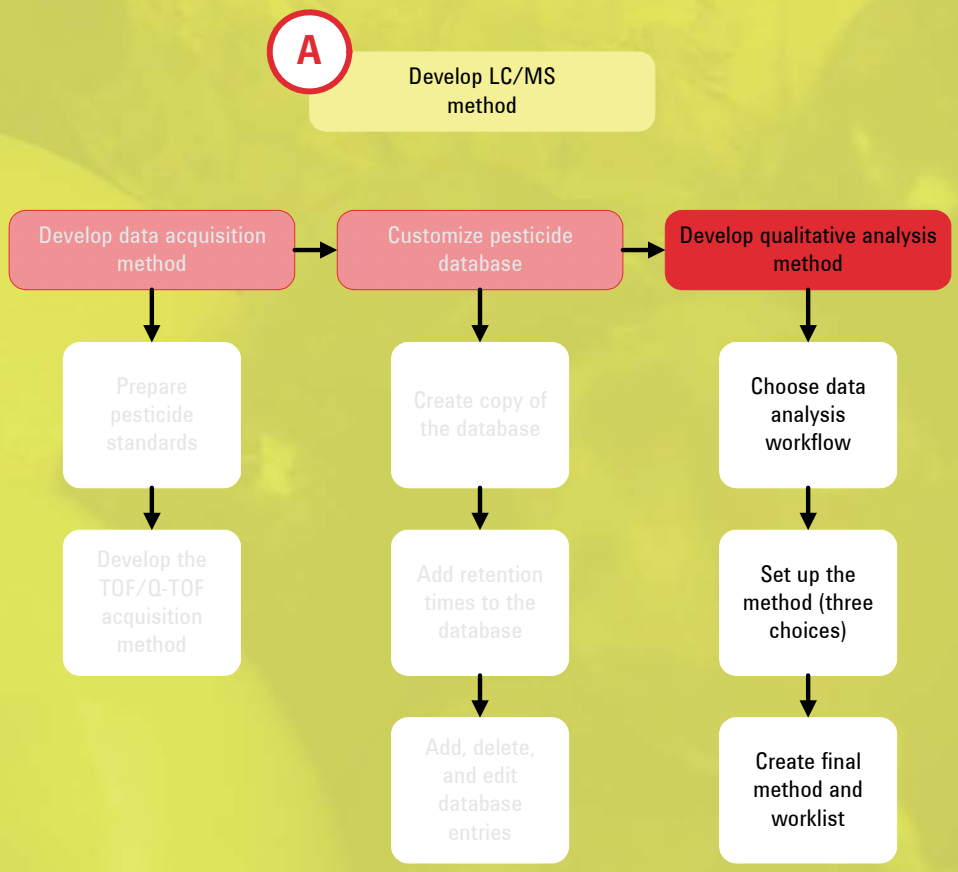
3. Edit the database.

   - Follow the directions in the *Agilent G6854 MassHunter Personal Pesticide Database Quick Start Guide* (Agilent publication G6854-90003, First Edition, July 2009).
   - In the "Familiarization Exercises" section, see "Exercise 4. Edit compounds." Start with step 2.

   If you need to import a large list of compounds, in this Quick Start Guide, see "To import compounds to a custom database" in the section on "Database operations."

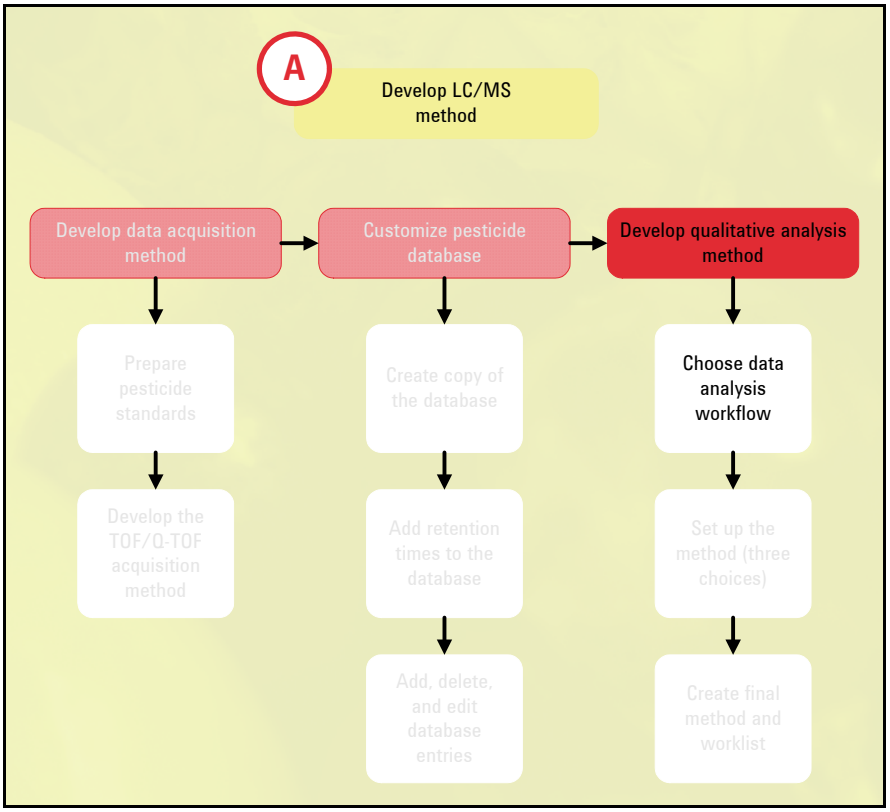
Developing the Qualitative Analysis Method

These exercises show you how to set up a Qualitative Analysis method for multi-residue pesticide screening with an Agilent TOF or Q-TOF and MassHunter Workstation software.



Choose a data analysis workflow

In this exercise, you choose one of three workflows for your data analysis method. For all workflows, MassHunter Qualitative Analysis uses the MassHunter Personal Pesticide Database (or a custom pesticide database) to find the pesticides in the sample.



Learn about the workflows for Qualitative Analysis B.04.00

1. If you have Qualitative Analysis B.04.00, read the summaries here. If you have Qualitative Analysis B.05.00, go to the next section.

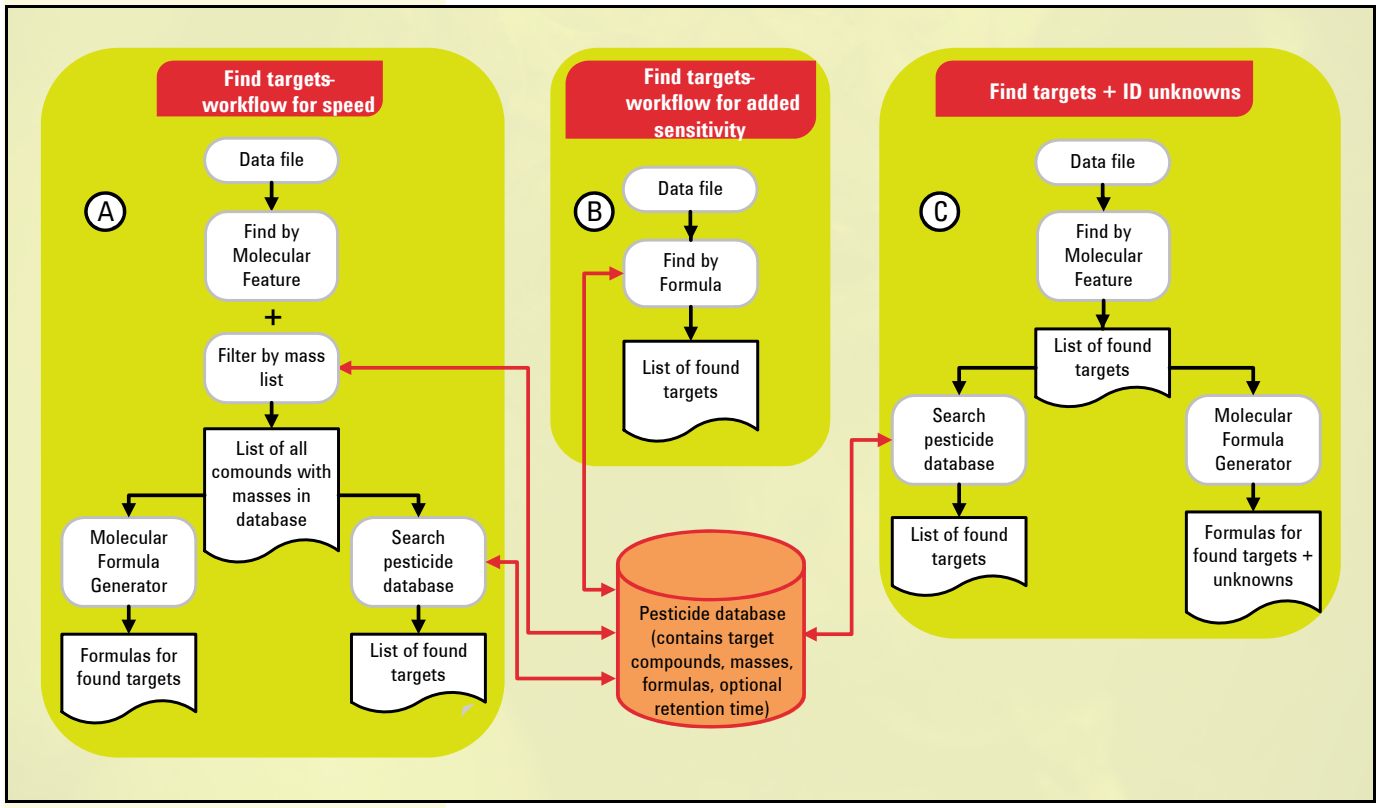


Figure 7 *Choice of workflows for data analysis*

The choice of workflow depends on the needs of your lab.

- *Method A* in Figure 7 uses molecular feature extraction to find compounds and then searches the found compounds against the pesticide database. Matches take into consideration both accurate masses and isotope information. This method is equivalent to a forward search. When you search a large database, this method is much faster in computation time than *Method B*, but it is more prone to false positives.

  Method A also includes molecular formula generation (MFG) for compounds that have masses in the database.

- *Method B* finds compounds by formula, where the formulae come from the pesticide database. Matches take into consideration both accurate masses and isotope information. This method is equivalent to a reverse search. It provides even greater sensitivity than Method A because it uses extracted ion chromatograms (EICs) to find the target compounds.

  If you need to analyze many hundreds of compounds and your custom database does *not* have retention times, consider using one of the other methods because they will be much faster than *Method B*. If you have 1600 pesticides in your database and no retention times, *Method B* is very slow. For more information, see "Settings to reduce processing time for Find by Formula" on page 81.

- *Method C* is similar to *Method A*, but also uses molecular formula generation (MFG) to calculate formulae of unknown compounds not found in the pesticide database.

---

2. Learn more about the data analysis approaches.

- Read "Increased Productivity for Target Compound Screening Using TOF-MS and Accurate-Mass Databases with Optional Retention Time – *Part 2*: Integrated, Automated Workflows" (Agilent technical note 5990-4829EN, December 2009).
- See especially the section on "Multiple workflows in MassHunter Qualitative Analysis."
- If you want to read Part I of this technical note, download "Increased Productivity for Target Compound Screening Using TOF-MS and Accurate-Mass Databases with Optional Retention Time – *Part 1*: Unique Capabilities of Agilent Software" (Agilent technical note 5990-4828EN, December 2009).

Note that developing experience with this type of screening is critical, and using the capability to *customize* and make a *personal* compound database is a key to successful targeted and nontargeted screening.

Learn about the workflows for Qualitative Analysis B.05.00

1. If you have Qualitative Analysis B.05.00, read the summaries here. If you have Qualitative Analysis B.04.00, read the summaries in the previous section.

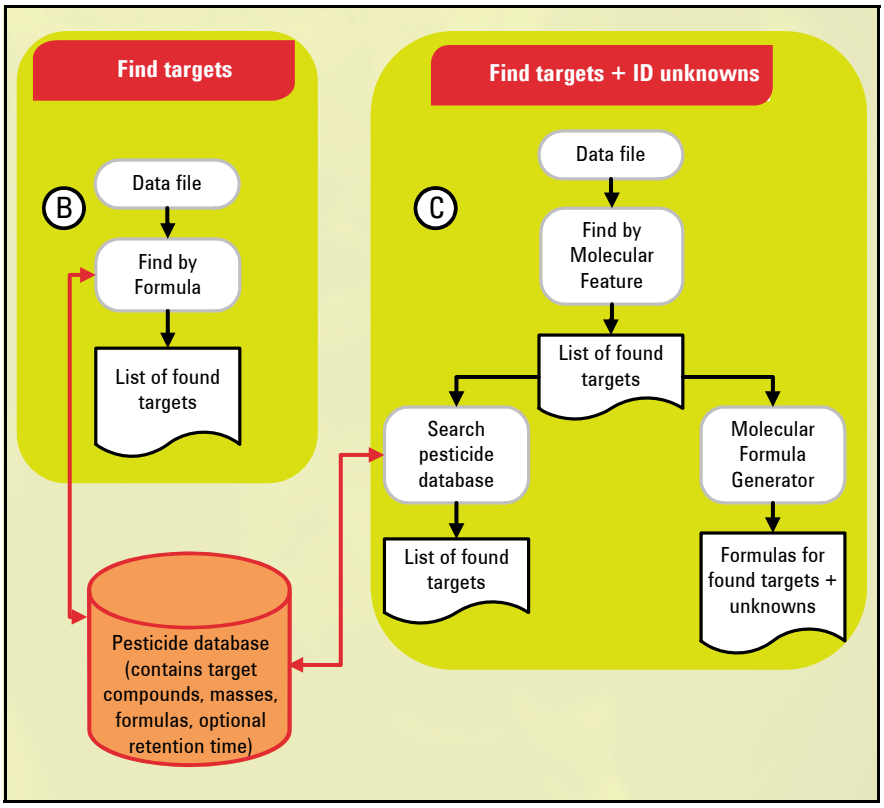


Figure 8 *Choice of workflows for data analysis*

The choice of workflow depends on the needs of your lab.

- *Method B* in Figure 8 finds compounds by formula, where the formulae come from the pesticide database. Matches take into consideration both accurate masses and isotope information. This method is equivalent to a reverse search.

  Starting with Agilent MassHunter Qualitative Analysis B.05.00, Find by Formula is significantly faster, making Method B an excellent choice for pesticide screening.

- *Method C* uses molecular feature extraction to find compounds and then searches the found compounds against the pesticide database. Matches take into consideration both accurate masses and isotope information. This method is equivalent to a forward search. This method also uses molecular formula generation (MFG) to calculate formulae of unknown compounds not found in the pesticide database.

2. Learn more about the data analysis approaches.

- Note that developing experience with compound screening is critical, and using the capability to *customize* and make a *personal* compound database is a key to successful targeted and nontargeted screening.

Choose a workflow

1. Consider your lab's needs.

- Choose a suitable approach based on:
  - Whether you need to identify nonpesticide sample components
  - Your tolerance for false positives and false negatives

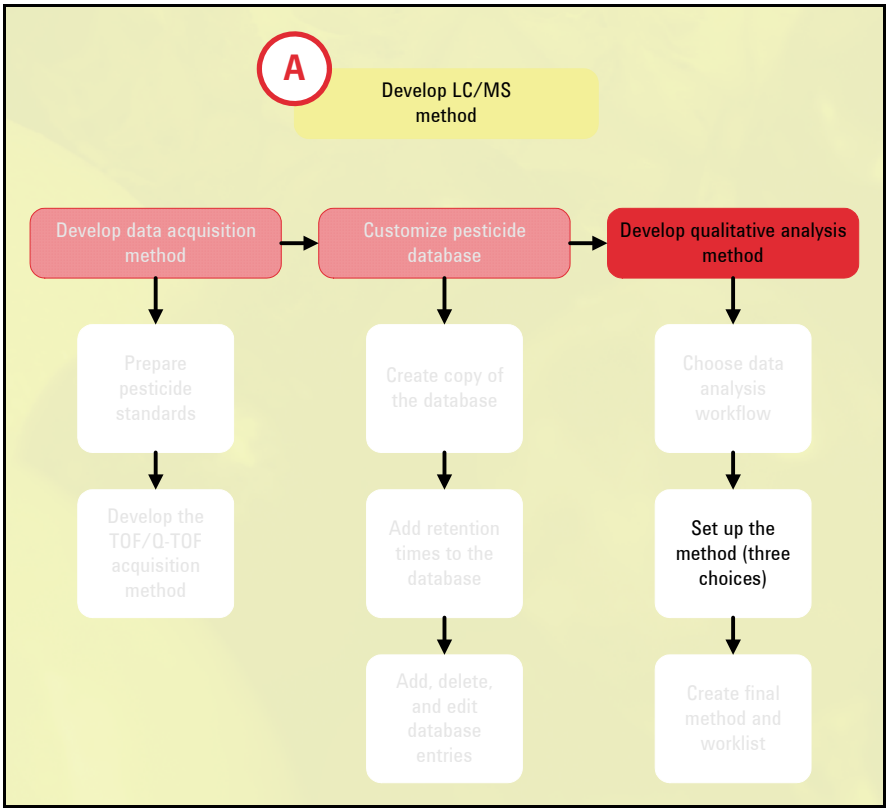
2. Pick a workflow.

Pick a workflow based on Figure 8 and the descriptions under it.

Set up the data analysis method (three choices)

In this exercise, you develop a MassHunter Qualitative Analysis method for pesticide screening. You do only one of the three tasks in this section—the one that matches the approach you chose in the previous exercise. Depending on your software version, the previous exercise was either:

- "Learn about the workflows for Qualitative Analysis B.04.00" on page 39, or
- "Learn about the workflows for Qualitative Analysis B.05.00" on page 41.



For general background information about use of MassHunter Qualitative Analysis methods for compound screening, read:

- "An Application Kit for Multi-Residue Screening of Pesticides using LC/TOF or Q-TOF with a Pesticide Personal Compound Database" (Agilent application note 5990-4251EN, August 2009)
- "An Application Kit for the Screening of Samples for Analytes of Forensic and Toxicological Interest using TOF or Q-TOF LC/MS with a Personal Forensics/Toxicology Database" (Agilent application note 5990-4252EN, August 2009)

To set up the method for Find by Molecular Feature Extraction (MFE) (Method A in Figure 7)

In this task, you set up a method to find all the compounds in a data file, then filter the masses of the found compounds by the monoisotopic masses in the pesticide database. You set up the method to then search the filtered compounds against the pesticide database.

You also set the method to generate molecular formulae for compounds that have database matches. You can then compare database search results with the generated formulae, which gives greater confidence in valid results and alerts you when an alternative formula is possible. For information about Agilent molecular formula generation (MFG), see:

- "Superior Molecular Formula Generation from Accurate-Mass Data" (Agilent technical note 5989-7409EN, January 2008)

The PCDL Manager software scores the database search results by how well the following parameters match between the experimentally measured results and the calculated result for the database entry:

- Accurate monoisotopic mass
- Isotope ratio
- Isotope spacing

This unique scoring algorithm increases confidence in the results.

1. Open MassHunter Qualitative Analysis.

   - Double-click the MassHunter Qualitative Analysis icon.

2. Use the MS Target Compound Screening workflow.

   a Click **View > Configure for Workflow > MS Target Compound Screening**.

   b If you see a message that describes what the program will do next, click **OK**.

   This step is important because it loads the method **screening-default.m**. If you start with a different method, these instructions will not be correct.

3. Save the method with a new name.

   a Click **Method > Save As**.

   b Give the method a new name (for example, **Screening-Pesticide-MFE.m**) and click **OK**.

4. Open a data file and observe the spectral noise level.

   a Click **File > Open Data File**.

   b Select the data file that you created in "Acquire data for test mixture of pesticides" on page 19 and click **OK**.

   c Display a spectrum.

   d Determine the approximate noise level. You need this number in the next step. For details on how to determine the noise level in spectra, see page 70.

5. Set parameters for Find by Molecular Feature – Extraction tab.

   a In Method Explorer, click **MS Target Compound Screening Workflow > Find by Molecular Feature**.

   b Click the **Extraction** tab.

c Under Peak filters, set **Use peaks with height** to 3X the noise level you determined in the previous step.

If you change the MS data acquisition method, change the setting for **Use peaks with height** to match the new noise level.

For more information about settings for MFE, as well as figures that show initial settings, see "Initial settings for Find by Molecular Feature Extraction" on page 69.

6. Set parameters for Find by Molecular Feature – Ion Species tab.

a Click the **Ion Species** tab.

b Mark the adducts you expect to see.

7. Set parameters for the Compound Filters and Mass Defect tabs.

a Click each tab.

b Make sure no filters are set.

8. Set parameters for Find by Molecular Feature – Mass Filters tab.

a Click the **Mass Filters** tab.

b Mark the check box for **Filter mass list**.

c Set the filter to **3 ppm**, or the mass accuracy you routinely observe in complex matrices.

d Select **Include only these mass(es)**.

e Click **Database**.

f Under Database, replace **default.csv** with **Pesticides.cdb** or a custom database.

If you wish to also generate formulae for unknown compounds *not* found in the database (as in Method C in Figure 7 on page 39), do *not* filter the mass list by masses in the database. Clear the check box for **Filter mass list**, or mark the check box and set up to *exclude* background masses.

9. Set parameters for Find by Molecular Feature – Results tab.

a Click the **Results** tab.

b Set it up as shown in Figure 18 on page 73.

10. Set parameters for Find by Molecular Feature – Charge State tab.

a In Method Explorer, click **Find Compounds > Find by Molecular Feature**.

b Click the **Charge State** tab.

c Mark **Limit assigned charge states to a maximum of** and type `1`.

d Mark **Treat ions with unassigned charge as single-charged**.

11. Set parameters for database search.

a In Method Explorer, click **MS Target Compound Screening Workflow > Identify by Database Search**.

b In the Database tab, replace **default.csv** with **Pesticides.cdb** or your custom database. Hint: To open ***.cdb** files, in the Open Database File dialog box, change **Files of type** to **PCDL Files (*.cdb)**.

c Click the **Search Criteria** tab.

d Do one of the following:
- If you want to find both targeted and nontargeted pesticides, click **Mass and retention time (retention time optional)**.
- If you want to find targeted pesticides only, click **Mass and retention time (retention time required)**.

e Under Match tolerance:
- Set **Mass** to **3 ppm** (or the mass accuracy you routinely observe in complex matrices). Use the same tolerance (ppm) as you used in step 8 above.
- Set **Retention time** to **0.1 min** (or the variation you expect in retention time). Note that this is a ± setting. A value of 0.1 min sets a 0.2-min retention time window.

---

12. Set the report options.

a In Method Explorer, click **MS Target Compound Screening Workflow > Compound Report**.

b Click the **Templates** tab.

c For Compound report template, select **CompoundReportWithIdentification-Hits.xltx**.

d Click the **Worklist Options** tab.

e Set up the type of report you want (printed, Microsoft® Excel, or Adobe® PDF).

It is important to use the **CompoundReportWithIdentificationHits.xltx** report template because if multiple database hits exist, this report shows them all.

If you want to customize the report, see the following for instructions:
- *Agilent MassHunter Workstation Software Reporting Familiarization Guide* (Agilent publication G3335-90110, First Edition, July 2011)).
- *Agilent MassHunter Reporting User Information DVD* (Agilent publication G6845-60007, May 2011).

---

13. Set parameters for automation.

a In Method Explorer, click **MS Target Compound Screening Workflow > Automation**.

b Click the **Analysis Options** tab.

c Click **Find by Molecular Feature**.

d Confirm that the check box for **Search a database for each compound** is marked.

e Mark the check box for **Generate formulas for each compound**. (You can then compare database search results with the generated formulas,)

f Click **All Compounds**.

g Mark the check box for **Show only identified compounds**.

14. Save the method.

- Click **Method > Save**.

15. Test the method.

a Click **Actions > Run the Worklist Actions**.

b Verify that you receive the desired report and that the results are reasonable.

c To evaluate the MFE results, check some of the compounds found at lower levels:
- In the Compound Table, right-click a low-level compound and click **Extract complete result set**.
- Compare the EICs and the ECCs. If they match fairly well, the settings are probably good. If they do not, adjust the MFE settings, as described in the next step.

16. Adjust the method, if necessary.

a In Method Explorer, click **MS Target Compound Screening Workflow > Find by Molecular Feature**.

b Click the **Extraction** tab.

c Under Peak filters, set **Use peaks with height** to a new noise value (likely higher to be more selective).

d Save the method.

A higher threshold reduces the number of false positives, but you may miss some low-level pesticides that are present.

17. Test the method again, and save when you are done.

a Click **Actions > Run the Worklist Actions**.

b Evaluate the results.

c Make further adjustments if necessary.

d Save the method when you are done.

To set up the method for Find by Formula (Method B in Figure 7 and Figure 8)

In this task, you set up a method to search a data file for the accurate masses of all pesticides in a pesticide database. This method can find pesticides at very low concentrations, but (if you have MassHunter Qualitative Analysis B.04.00) it takes longer than Find by Molecular Feature—the method in the previous task. If you have MassHunter Qualitative Analysis B.04.00 and you need to reduce processing time for Find by Formula, see "Initial settings for Find by Formula" on page 75.

Find by Formula compares the experimentally measured results and the calculated result for the database entries. It scores the results by how well the following parameters match:

- Accurate monoisotopic mass
- Isotope ratio
- Isotope spacing

1. Open MassHunter Qualitative Analysis.

   - Double-click the MassHunter Qualitative Analysis icon.

2. Use the MS Target Compound Screening workflow.

   a Click **View > Configure for Workflow > MS Target Compound Screening**.

   b If you see a message that describes what the program will do next, click **OK**.

3. Save the method with a new name.

   a Click **Method > Save As**.

   b Give the method a new name (for example, **Screening-Pesticide-FindByFormula.m**) and click **OK**.

4. Open a data file.

   a Click **File > Open Data File**.

   b Select the data file that you created in "Acquire data for test mixture of pesticides" on page 19 and click **OK**.

5. Set parameters for Find by Formula – Formula Source tab.

   a In Method Explorer, click **MS Target Compound Screening Workflow > Find by Formula**.

   b In the Formula Source tab, make sure **Database** is selected, then replace **default.csv** with **Pesticides.cdb** or a custom database. (Hint: To open ***.cdb** files, in the Open Database File dialog box, change **Files of type** to **PCDL Files (*.cdb)**.

   c For Database values to match, do one of the following:
   - If you want to find both targeted and nontargeted pesticides, click **Mass and retention time (retention time optional)**.
   - If you want to find targeted pesticides only, click **Mass and retention time (retention time required)**.

For additional information and figures that show settings, see "Initial settings for Find by Formula" on page 75.

See also the tips in "Settings to reduce processing time for Find by Formula" on page 81.

6. Set parameters for Find by Formula – Formula Matching tab.

a Click the **Formula Matching** tab.

b Under Match tolerance:
- Set **Masses** to **± 3 ppm**, or the mass accuracy you typically observe in complex matrices.
- Set **Retention times** to **± 0.1 min**.

c Under Expansion of values for chromatogram extraction:
- Set **Possible m/z** to **Symmetric (ppm)**, with a window of **± 20**.
- Set **Expected retention time** to **± 1.0 min**.

The 0.1-min window in step b is used by the database search to exclude isomers that elute close together, while the 1.0-min retention time window in step c is used for chromatogram extraction and signal-to-noise calculation.

7. Set parameters for Find by Formula – Positive Ions or Negative Ions tab.

a Depending on the polarity of your runs, click either the **Positive Ions** tab or the **Negative Ions** tab.

b Mark check boxes for only the adducts you expect to see.

c Set **Charge state range** to **1**.

8. Set parameters for Find by Formula – EIC Peak Filters tab.

a Click the **EIC Peak Filters** tab.

b Under Absolute filters, set **Absolute area** to a good starting value. To determine the appropriate number, sort the compound results by peak area. (Right-click in the Compound List and add the **Area** column if it is not already present.) Review the EICs, note a peak area cutoff that generates acceptable results (not noise), and set the value to that number.

9. Set parameters for Find by Formula – Results tab.

a Click the **Results** tab.

b Set it up as shown in Figure 25 on page 80.

10. Set the report options.

a In Method Explorer, click **MS Target Compound Screening Workflow > Compound Report**.

b Click the **Templates** tab.

c For Compound report template, select **CompoundReportWithIdentification-Hits.xltx**.

d Click the **Worklist Options** tab.

e Set up the type of report you want (printed, Excel, or PDF).

It is important to use this report template because if multiple database hits exist, this report shows them all.

If you want to customize the report, see the following for instructions:
- *Agilent MassHunter Workstation Software Reporting Familiarization Guide* (Agilent publication G3335-90110, First Edition, July 2011)
- *Agilent MassHunter Reporting User Information DVD* (Agilent publication G6845-60007, May 2011)

11. Set parameters for automation.

a In Method Explorer, click **MS Target Compound Screening Workflow > Automation**.

b Verify that **Find by Formula** is selected.

c Clear the check boxes for **Search a database for each compound** and **Generate formulas for each compound**.

12. Save the method.

- Click **Method > Save**.

13. Test the method.

a Click **Actions > Run the Worklist Actions**.

b Verify that you get the desired report and that the results are reasonable.

c If you get noisy results, go to the next step.

The algorithm assesses the chromatographic peak shape and isotopic match scores and returns the best match, even if several peaks are displayed in the EIC.

14. Adjust the method, if necessary.

a In Method Explorer, click **MS Target Compound Screening Workflow > Find by Formula**.

b Change the appropriate parameters.

c Save the method.

For guidance, see the tips in "Initial settings for Find by Formula" on page 75 and "Settings to reduce processing time for Find by Formula" on page 81.

15. Test the method again, and save when you are done.

a Click **Actions > Run the Worklist Actions**.

b Evaluate the results.

c Make further adjustments if necessary.

d Save the method when you are done.

To set up the method for Find by MFE plus formula generation for unknowns (Method C in Figure 7 and Figure 8)

In this task, you set up a method to find all the compounds in a data file, then search the compounds against the pesticide database. You also set the method to generate molecular formulae for both compounds that were found in the database and unknowns that were not found. For information about Agilent molecular formula generation (MFG), see:

- "Superior Molecular Formula Generation from Accurate-Mass Data" (Agilent technical note 5989-7409EN, January 2008)

For additional confidence, both the database search scores and the MFG scores take into account:

- Accurate monoisotopic mass
- Isotope ratio
- Isotope spacing

1. Open MassHunter Qualitative Analysis.

   - Double-click the MassHunter Qualitative Analysis icon.

2. Use the MS Target Compound Screening workflow.

   a Click **View > Configure for Workflow > MS Target Compound Screening**.

   b If you see a message that describes what the program will do next, click **OK**.

3. Save the method with a new name.

   a Click **Method > Save As**.

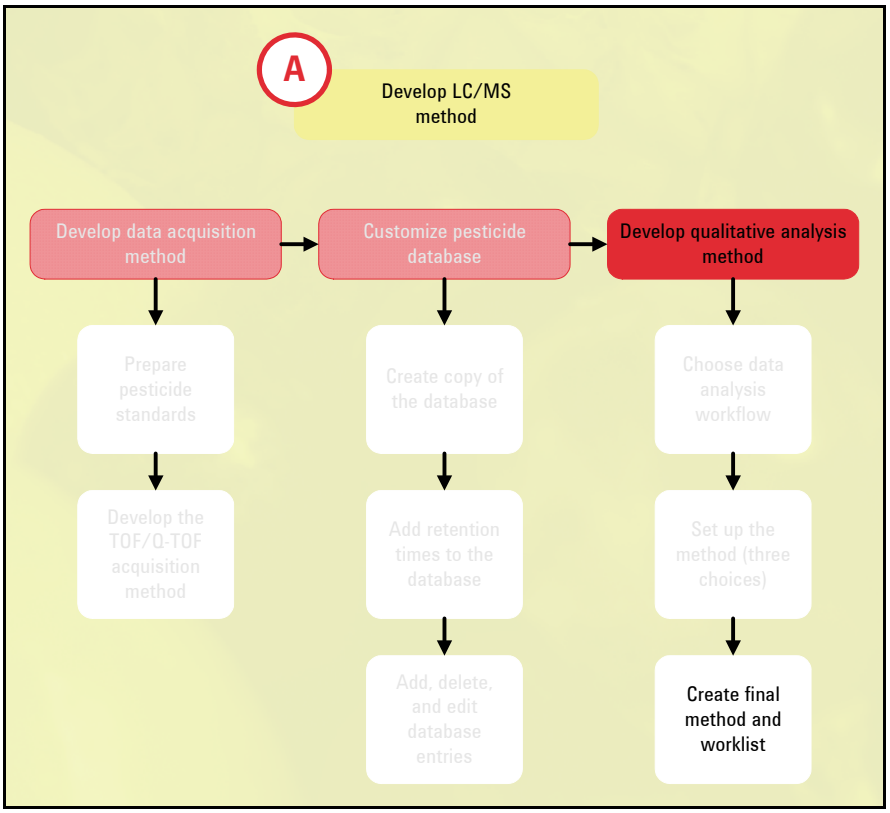
   b Give the method a new name (for example, **Screening-Pesticide-MFE+Unk.m**) and click **OK**.

4. Set up the method, similar to an earlier section.

   - Set up the method, similar to "To set up the method for Find by Molecular Feature Extraction (MFE) (Method A in Figure 7)" on page 44.
   - Follow step 4 through step 17 in that section, except do *not* do step 8 as described there. Instead, clear the check box for **Filter mass list**, or mark the check box and set up to *exclude* background masses.

Create final method and worklist

This exercise shows you how to combine a MassHunter Data Acquisition method with a MassHunter Qualitative Analysis method, for a completely automated analysis. You do this exercise regardless of whether you have MassHunter Qualitative Analysis B.04.00 or B.05.00.



The tasks below describe two ways to do this:

- Attach the MassHunter Qualitative Analysis method that you created in this chapter to the MassHunter Data Acquisition method that you developed in Chapter 2. Then run the combined method.
- Keep the two methods separate, then (in a worklist) override the data analysis portion of the MassHunter Data Acquisition method and run the desired MassHunter Qualitative Analysis method instead.

You choose which approach you prefer and do the corresponding task only.

To add the data analysis method to the data acquisition method

In this task, you combine your data acquisition and data analysis methods into a single method. You then set up a worklist to automate pesticide screening and reporting using the combined method.

1. Open your data analysis method.

   a Open MassHunter Qualitative Analysis.

   b Open the MassHunter Qualitative Analysis method that you created in this chapter.

2. Save the data analysis method within the data acquisition method.

   a In the MassHunter Qualitative Analysis program, click **Method > Save As**.

   b Browse to the folder that contains the data acquisition method that you created in Chapter 2.

   c Click the name of the data acquisition method and click **Save**. The data analysis method is now part of the data acquisition method.

3. In MassHunter Data Acquisition, create a worklist that runs the combined method.

   a Follow the directions in step 3 in "Analyze pesticides to get retention times" on page 27. Use the method you just created.

   b Click **Worklist > Worklist Run Parameters**.

   c Under Run Information, for **Part of method to run**, select **Both Acquisition and DA**.

   d For **Execution for Acquisition-DA**, do one of the following:
   - Select **Synchronous** if you want data analysis to finish before the next sample is injected.
   - Select **Asynchronous** if you want to inject the next sample without waiting for data analysis to finish.

   e Click **OK**.

4. Save the worklist.

   - Click **File > Save > Worklist**.

To run data acquisition and data analysis as separate methods

In this task, you keep separate the data acquisition and data analysis methods you have developed. You set up a worklist to automate pesticide screening and reporting with these two methods.

1. Set up a worklist that uses both methods.

   - Follow the directions in step 3 in "Analyze pesticides to get retention times" on page 27.
   - In the Sample Information tab, set up the worklist with the data acquisition method that you created in Chapter 2 and the data analysis method that you created in this chapter. See Figure 9.

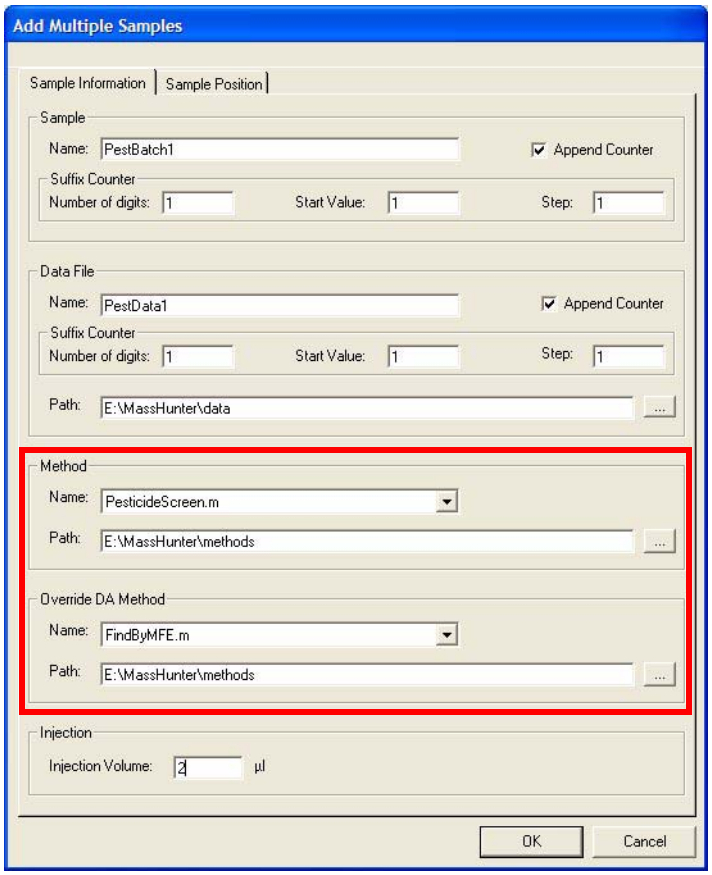


Figure 9 *Setup of separate data acquisition and data analysis methods*

If you do not see the column for **Override DA Method** in the worklist, it may be hidden between the **Method** and **Data File** columns. Move the mouse pointer to the boundary between these two columns. When the pointer changes to a double-sided arrow, move the column boundary to the right until you see the **Override DA Method** column. See Figure 10.

		Sample Name	Sample Position	Method	Override DA Method	Data File	Sample Type	Lev
1	✓	PestBatch11	P1-A1	PesticideScreen.m	FindByMFE.m	PestData11.d	Calibration	
2	✓	PestBatch12	P1-A2	PesticideScreen.m	FindByMFE.m	PestData12.d	Calibration	
3	✓	PestBatch13	P1-A3	PesticideScreen.m	FindByMFE.m	PestData13.d	Blank	
4	✓	PestBatch14	P1-A4	PesticideScreen.m	FindByMFE.m	PestData14.d	Sample	
5	✓	PestBatch15	P1-A5	PesticideScreen.m	FindByMFE.m	PestData15.d	Sample	
6	✓	PestBatch16	P1-B1	PesticideScreen.m	FindByMFE.m	PestData16.d	Sample	
7	✓	PestBatch17	P1-B2	PesticideScreen.m	FindByMFE.m	PestData17.d	Sample	
8	✓	PestBatch18	P1-B3	PesticideScreen.m	FindByMFE.m	PestData18.d	Sample	
9	✓	PestBatch19	P1-B4	PesticideScreen.m	FindByMFE.m	PestData19.d	Sample	
10	✓	PestBatch110	P1-B5	PesticideScreen.m	FindByMFE.m	PestData110.d	Sample	

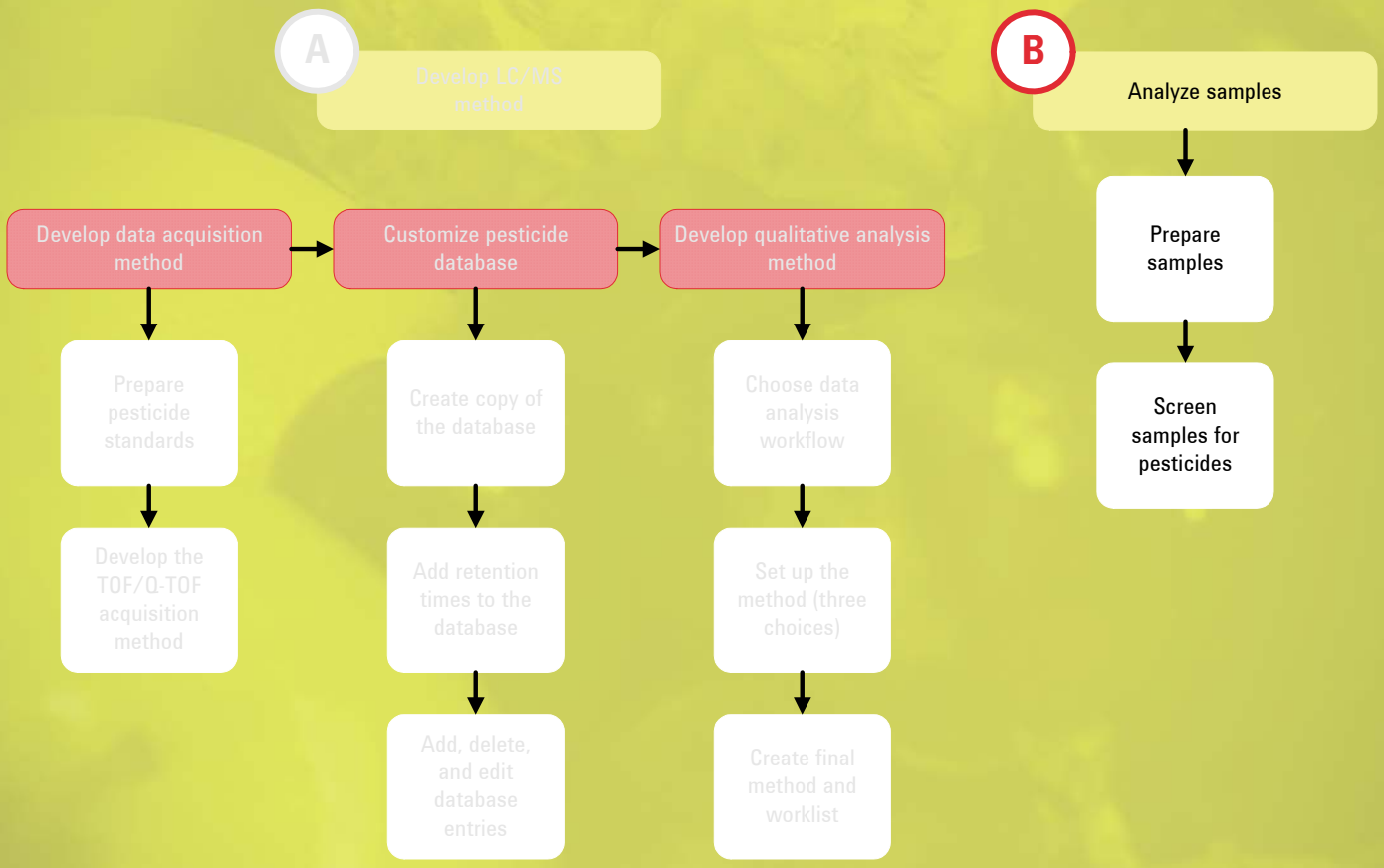
Figure 10 *Worklist showing separate data acquisition and data analysis methods. Note that you may need to move the column boundary to see the* ***Override DA Method*** *column*

2. Save the worklist.

- Click **File > Save > Worklist**.

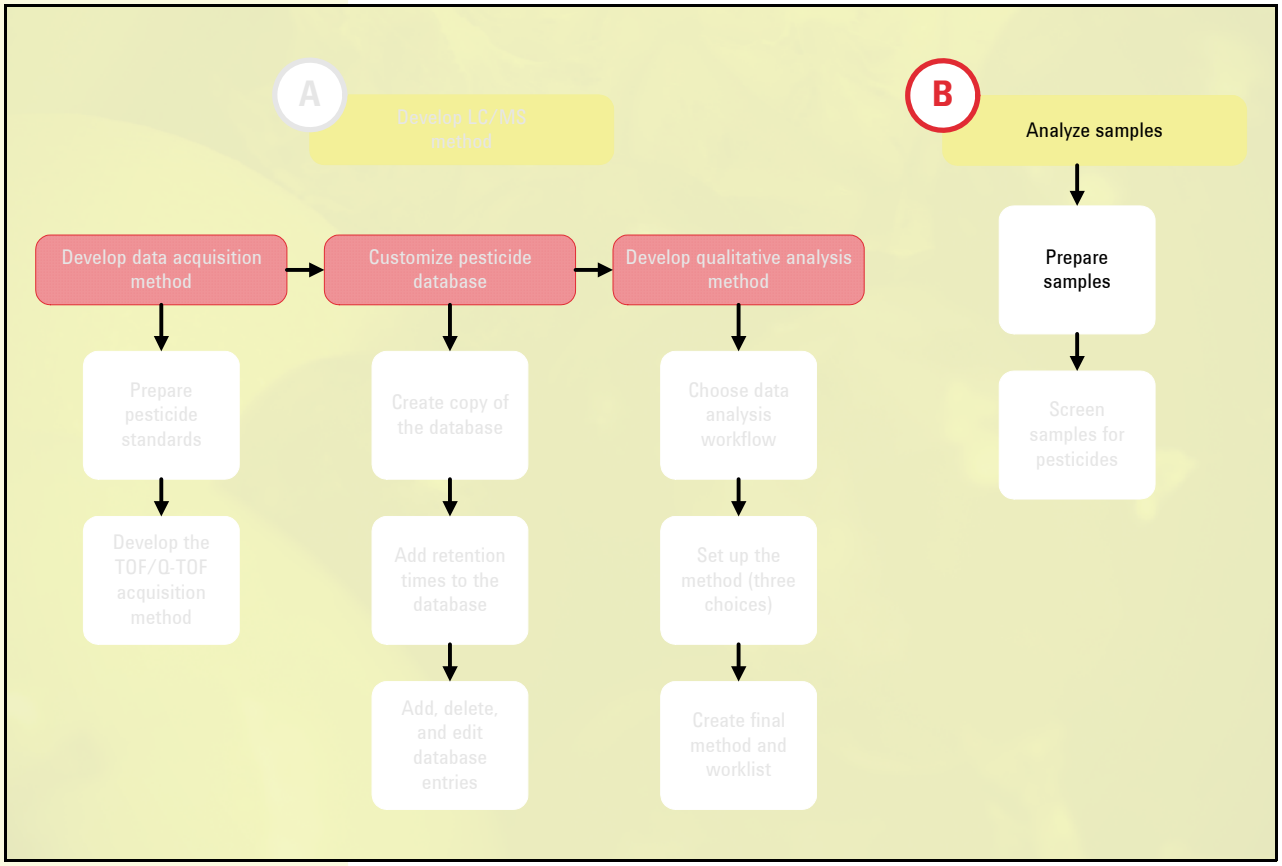
Analyzing Samples

This chapter describes how to prepare samples and analyze them for pesticides, using the MassHunter Data Acquisition and MassHunter Qualitative Analysis methods that you developed in previous chapters. The sample preparation method is specific to spinach, but the rest of the chapter about the LC/MS analysis applies to any sample matrix.



Prepare samples

In this exercise, you prepare a spinach sample using Agilent SampliQ QuEChERS extraction and sample cleanup kits. If you analyze other foods, your sample preparation may be similar. However, it is important to select the proper kit for each type of food sample. See "Agilent SampliQ QuEChERS Kits" (Agilent publication number 5990-3562EN, February, 2010) for guidance. This publication also gives examples of sample preparation methods.



You can get more information about SampliQ QuEChERS on the Agilent Web site, including a standard operating procedure and a demo video to help you get started. An application compendium (5990-4977EN) describes food safety applications, with an emphasis on pesticide analysis.

If you analyze other matrices, such as soil or water, you need a different sample preparation method than the one given here.

Be sure to prepare any necessary QC samples and blanks along with your other samples. For examples, see the list to the right of step 1 on page 59.

Figure 11 *QuEChERS stands for **qu**ick, **e**asy, **ch**eap, **e**ffective, **r**ugged and **s**afe. These adjectives describe this sample preparation technique for multi-residue pesticide analysis in fruits and vegetables.*

1. Weigh samples.

- Weigh 15 g (±0.1 g) of homogenized spinach sample.

2. Spike samples.

- Spike samples as necessary with:
  - Standards to determine recoveries
  - Standards of targeted pesticides at the desired detection limit (to ensure that they are detected)
  - Internal standard solution

3. Vortex 30 sec.

4. Add 15 mL of 1% acetic acid in acetonitrile.

5. Add extraction packet.

- To each tube, add an Agilent SampliQ QuEChERS AOAC buffered extraction salt packet (from Agilent p/n 5982-5755).

Each packet contains 6 g anhydrous $MgSO_4$ and 1.5 g anhydrous sodium acetate.

6. Cap and hand-shake vigorously for 1 min.

Some Agilent SampliQ QuEChERS kits contain ceramic homogenizers, which make the shaking step easier and more consistent.

7. Centrifuge at 4000 rpm for 5 min.

8. Transfer sample extract to cleanup kit.

- Transfer upper layer to dispersive SPE kit for highly pigmented fruits and vegetables:
  - 1 mL to p/n 5982-5321 *or*
  - 8 mL to p/n 5982-5356

9. Vortex 1 min.

10. Centrifuge.

- Centrifuge 2-mL tubes at 13000 rpm for 2 min, or 15-mL tubes at 4000 rpm for 5 min.

11. Transfer upper layer.

- Transfer 200 µL of the upper layer to an autosampler vial.

12. Add water.

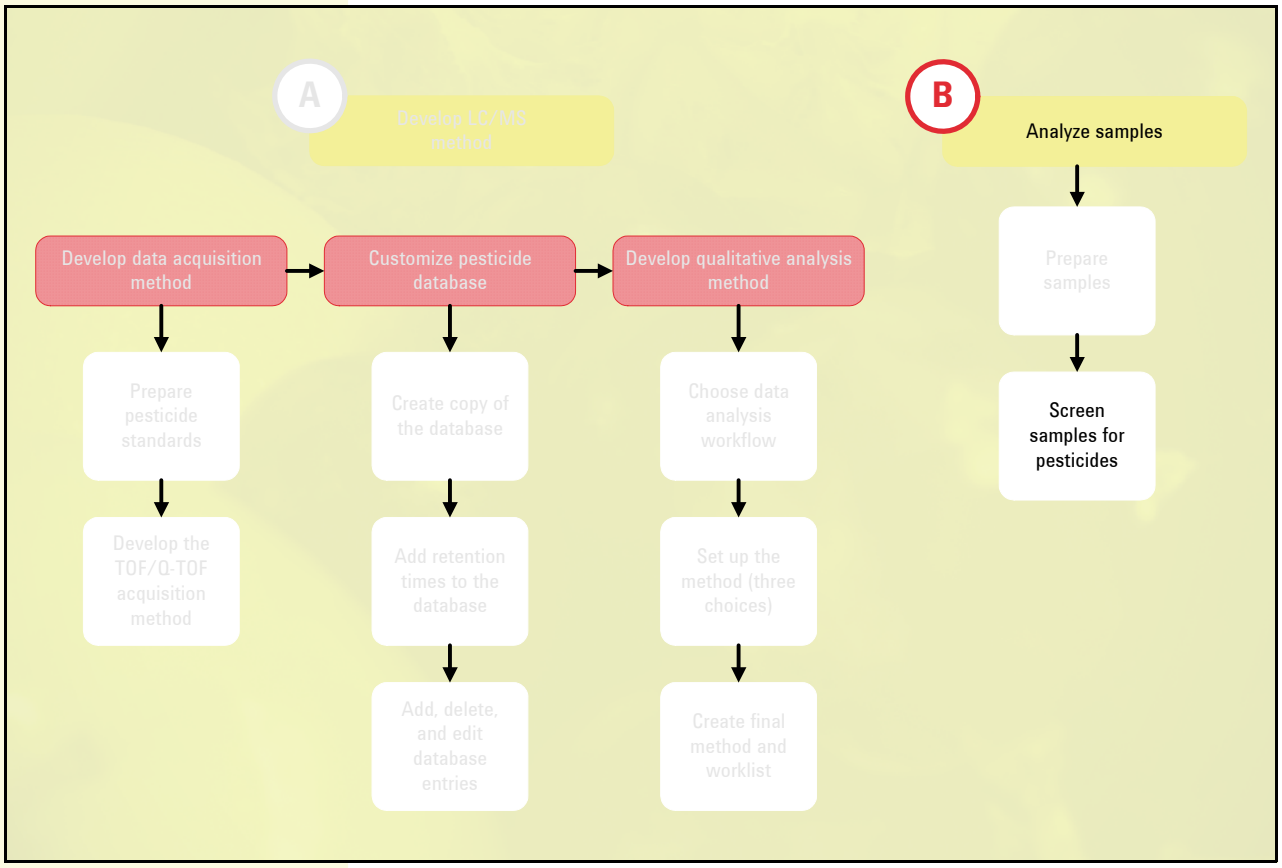
- Add 800 µL of water or appropriate standard spiking solution.

13. Cap and vortex.

- Cap the sample and vortex 1 min, to prepare for LC/MS analysis.

Screen samples for pesticides

In this exercise, you learn to do TOF or Q-TOF LC/MS analyses of samples and standards.



1. Make sure you have all the standards and samples you need.

- Make sure you have the following prepared in the appropriate containers for your LC autosampler:
  - Pesticide standards and/or QC samples to verify method performance
  - Sample extracts
  - Solvent blanks
  - Double blanks (no internal standard)

Analyze appropriate standards and QC samples to meet requirements of regulatory agencies and standard operating procedures for your lab. Make sure that you validate your method.

For quality control, Agilent also suggests zero control samples (samples known to be pesticide-free), to check for possible contamination in the sample preparation process.

2. Put the standards and samples into the LC autosampler.

3. Start the MassHunter Data Acquisition program.

- Double-click the Data Acquisition icon on your desktop.

4. Set up a worklist to perform a completely automated screening analysis.

a If necessary, click the **Worklist** button on the toolbar to display the Worklist pane. 

b To set up the worklist run, click **Worklist > Worklist Run Parameters**.

c Set the **Data File Path**, verify that the other parameters are set properly, and click **OK**.

d Click **Worklist > Add Multiple Samples**.

e Type or select the necessary information in the Sample Information tab. Be sure to select the correct method(s).

f Click the **Sample Position** tab.

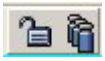
g Drag to select the sample positions, then click **OK**.

h Verify that the worklist has been populated with samples.

i Edit the worklist table as necessary. Be sure that it is set to run both data acquisition and data analysis, using the methods you developed in earlier exercises.

If you need practice to run a worklist, see the following:
- End of step 4 in the "Getting Started" section of the *Agilent 6200 Series TOF and 6500 Series Q-TOF LC/MS System Quick Start Guide*
- Task 2 in the "Set up and run single samples and worklists" section of the *Agilent MassHunter Workstation Software – Data Acquisition for 6200 Series TOF and 6500 Series Q-TOF Familiarization Guide* (Agilent publication G3335-90066, Third Edition, May 2009)

5. Run the analysis.

a To start the run, click the **Run Worklist** button on the toolbar. 

b Wait for the worklist to finish.

c Verify that your analysis was successful. See Figure 12 as an example.

You can run the worklist in either locked or unlocked mode. When the mode is locked, no one can change the method or the worklist while the worklist is running.

6. Review the reports that are automatically generated.

a Verify that any standards and QC samples gave appropriate results.

b Review the sample data to ensure that the data acquisition and data analysis worked properly.

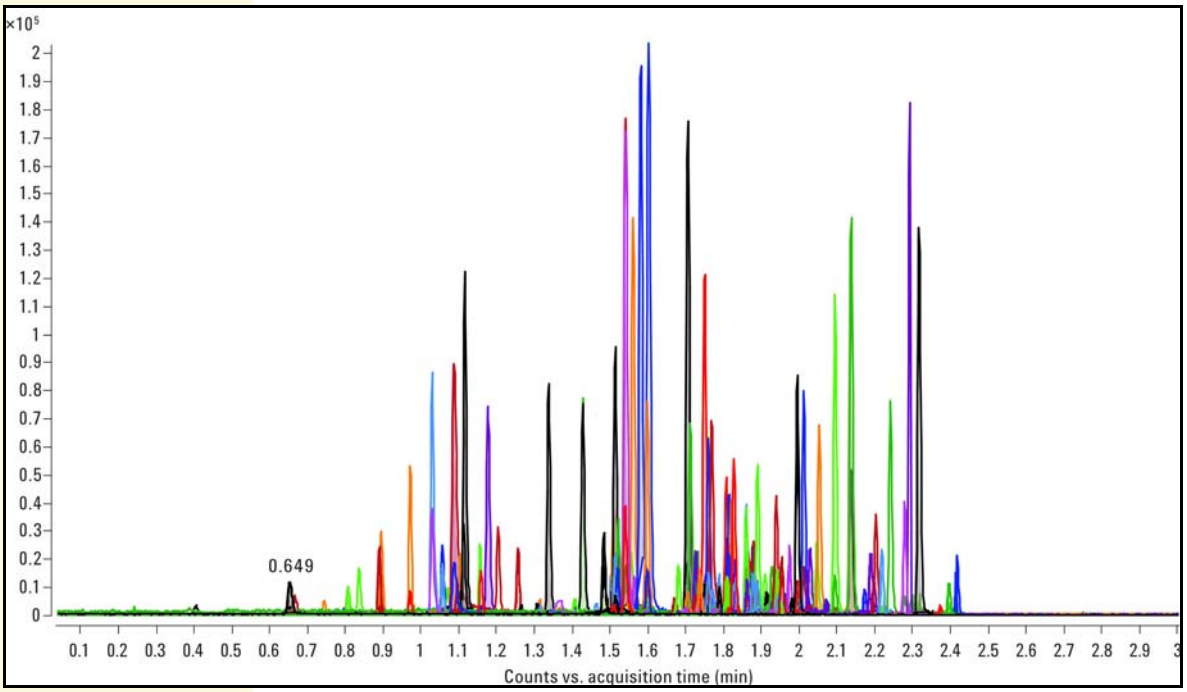
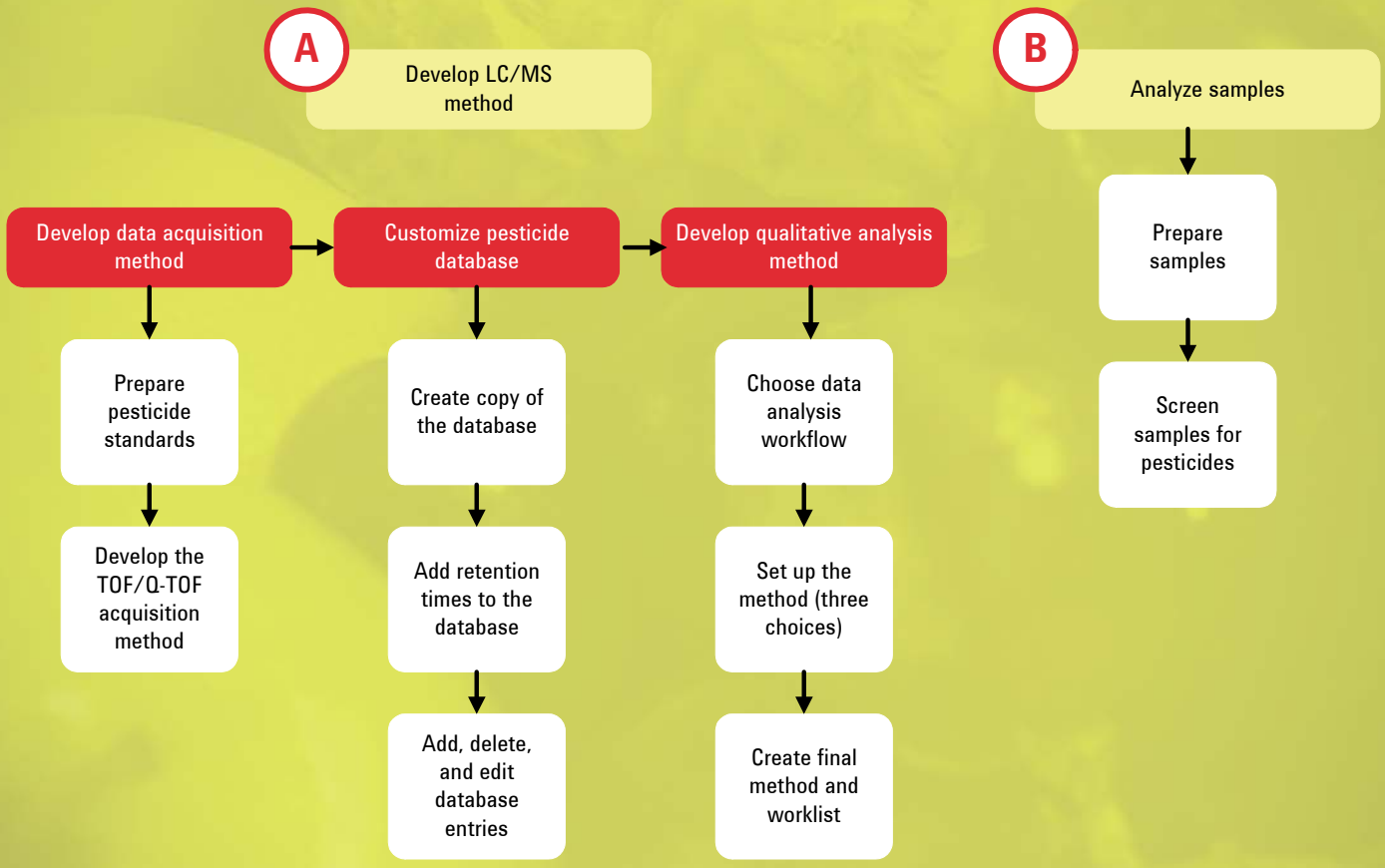


Figure 12 *This example shows an extracted compound chromatogram (ECC) from a TOF analysis of 100 pesticides in 3 min. This analysis used an Agilent 1290 Infinity LC System and an Agilent 6540 Ultra High Definition Q-TOF LC/MS System.*

Reference Information

This chapter lists the lab equipment, lab supplies, and chemicals you need to do these analyses. It provides LC/MS settings, as well as tips to optimize data analysis. It also lists manuals, application and technical notes, and other reference materials that will help you to be successful with your analyses.



Required supplies and chemicals

Required equipment and lab supplies

The lab equipment, solvents, and chemicals that you need will depend on the sample matrix and pesticides to be analyzed. The following sections give general guidelines.

Description	Vendor and part number
Analytical balance	
Spatula, to weigh standards	
Weigh boats or paper	
Protective gloves	
Safety glasses	
Lab coat or other protective clothing	
Fume hood	
Volumetric flasks, for standard preparation	
Pipetman micropipettors, (P-10, P-20, P-200, P-1000) or equivalent	•Gilson
Vials to store pesticide standards, for example: •2-mL amber vials, 100/pk •Blue screw caps (for 2-mL vials), 100/pk, PTFE/silicone/PTFE septa	 •Agilent p/n 5182-0716 •Agilent p/n 5182-0723
Refrigerator for flammables storage (to store pesticide standards)	
Containers for LC autosampler	
Analytical LC column, for example: •Agilent ZORBAX Eclipse Plus C18 Rapid Resolution High Throughput (RRHT) column, 2.1 × 100 mm, 1.8 µm •Agilent ZORBAX Eclipse Plus C18 Rapid Resolution High Definition (RRHD) column, 2.1 × 100 mm, 1.8 µm •Agilent ZORBAX Eclipse Plus C18 RRHD column, 2.1 × 150 mm, 1.8 µm	 •Agilent p/n 959764-902 •Agilent p/n 959758-902 •Agilent p/n 959759-902
Container to capture LC waste (for example, old solvent bottle in secondary containment)	
SampliQ QuEChERS Buffered Extraction Kit, AOAC Method (used for analysis of pesticides in spinach)	•Agilent p/n 5982-5755
Centrifuge (used for analysis of pesticides in spinach)	
Vortex mixer	
SampliQ QuEChERS Dispersive SPE Cleanup Kit, EN Method (used for analysis of pesticides in spinach, not for use with planar pesticides)	•Agilent p/n 5982-5321 *or* 5982-5356
Nitrogen gas, for MS	

Required chemicals – solvents, reagents, and standards

Description	Vendor and part number
Pesticide standards	
Solvents to prepare pesticide standards, pesticide or LC/MS grade recommended	
LC solvents (for example, acetonitrile and/or methanol), pesticide or LC/MS grade recommended	
Milli-Q water or equivalent	
Glacial acetic acid, 99.9% (highest purity)	
Formic acid (highest purity)	
Ammonium acetate (highest purity)	
Ammonium formate (highest purity), for example: •5 M ammonium formate	•Agilent p/n G1946-85021

Optional equipment and chemicals

Description	Vendor and part number
Ultrasonic bath	

Example LC/MS methods

This section provides examples of LC/MS settings for this workflow. These settings are taken from an Agilent publication:

- "An Application Kit for Multi-Residue Screening of Pesticides using LC/TOF or Q-TOF with a Pesticide Personal Compound Database" (Agilent application note 5990-4251EN, August 2009)

Settings for test mix samples – positive and negative ion

These conditions were chosen for the test mixes that are included with the Agilent MassHunter Personal Pesticide Database Kit (G6854AA), but may apply for other samples as well.

Parameter	Setting
Parameters for Agilent 1260 Infinity LC or Agilent 1200 Series Rapid Resolution LC	
Column	Agilent ZORBAX Eclipse Plus C18 RRHT, 2.1 × 100 mm, 1.8 µm Agilent p/n 959764-902
Column temperature	35 °C
Injection volume	5 µL
Autosampler temperature	Ambient
Needle wash	5 sec with methanol
Mobile phase	A = 5 mM acetic acid in water B = 100% acetonitrile
Flow rate	0.3 mL/min
Gradient	5% B at 0 min to 95% B at 12 min
Stop time	12 min
Post time	3 min
Agilent 6530 Q-TOF parameters, Agilent Jet Stream conditions	
Drying gas temperature	250 °C
Drying gas flow	7 L/min
Nebulizer pressure	40 psi
Sheath gas temperature	325 °C
Sheath gas flow	11 L/min
Capillary + ion	3500 V
Nozzle voltage	0 V
Capillary – ion	2500 V
Nozzle voltage	1500 V
Acquisition mode	MS1
Min range	100 *m/z*
Max range	1100 *m/z*
Scan rate	1.4/sec
Reference masses, positive ion	121.050873: $[M+H]^+$ for purine 922.009798: $[M+H]^+$ for HP-921

Parameter	Setting
Reference masses, negative ion	119.0362: $[M–H]^-$ for purine 980.016375: $[M+C_2H_3O_2]^-$ for HP-921 acetate adduct

Settings for Agilent 1260 Infinity LC + 6230 TOF

These conditions were optimized for a 17-min analysis of 200 pesticides.

Parameter	Setting
Parameters for Agilent 1260 Infinity LC or Agilent 1200 Series Rapid Resolution LC	
Column	Agilent ZORBAX Eclipse Plus C18 RRHT, 2.1 × 100 mm, 1.8 µm Agilent p/n 959764-902
Column temperature	55 °C
Injection volume	5 µL
Autosampler temperature	6 °C
Needle wash	Flushport (methanol: water 75:25), 5 sec
Mobile phase	A = Water with 5 mM ammonium formate + 0.01% formic acid B = 5 mM ammonium formate + 0.01% formic acid in 95:5 acetonitrile:water
Flow rate	0.3 mL/min
Gradient	6% B at 0 min 6% B at 0.5 min 95% B at 14 min 95% B at 17 min
Stop time	17 min
Post time	3 min
Agilent 6230 TOF parameters, Agilent Jet Stream conditions	
Drying gas temperature	225 °C
Drying gas flow	9 L/min
Nebulizer pressure	25 psi
Sheath gas temperature	350 °C
Sheath gas flow	11 L/min
Capillary voltage	4500 V
Nozzle voltage	500 V
Acquisition mode	MS1
Min range	25 *m/z*
Max range	3200 *m/z*
Scan rate	3/sec
Reference masses, positive ion	121.050873: $[M+H]^+$ for purine 922.009798: $[M+H]^+$ for HP-921

Settings for Agilent 1290 Infinity LC + 6540 Q-TOF

These conditions were optimized for a 3-min analysis of 100 pesticides.

Parameter	Setting
Agilent 1290 Infinity LC parameters	
Column	Agilent ZORBAX Eclipse Plus C18 RRHD, 2.1 × 100 mm, 1.8 µm Agilent p/n 959758-902
Column temperature	60 °C
Injection volume	5 µL
Autosampler temperature	6 °C
Needle wash	Flushport (methanol: water 75:25), 5 sec
Mobile phase	A = Water with 5 mM ammonium formate + 0.01% formic acid B = 5 mM ammonium formate + 0.01% formic acid in 95:5 ace-tonitrile:water
Flow rate	1.0 mL/min
Gradient	6% B at 0 min 6% B at 0.15 min 95% B at 2.1 min 95% B at 3 min
Stop time	3 min
Post time	1 min
Agilent 6540 Q-TOF parameters, Agilent Jet Stream conditions	
Drying gas temperature	325 °C
Drying gas flow	8 L/min
Nebulizer pressure	60 psi
Sheath gas temperature	350 °C
Sheath gas flow	12 L/min
Capillary voltage	4000 V
Nozzle voltage	500 V
Acquisition mode	MS1
Min range	100 *m/z*
Max range	1000 *m/z*
Scan rate	10/sec
Reference masses, positive ion	121.050873: $[M+H]^+$ for purine 922.009798: $[M+H]^+$ for HP-921

Tips

Initial settings for Find by Molecular Feature Extraction

Extraction tab

The figures in this section show you starting parameters for molecular feature extraction. You will likely need to optimize the settings, as described in the following pages.

To view and change the settings, in the Method Explorer, click **MS Target Compound Screening Workflow > Find by Molecular Feature**. Then click the appropriate tab.

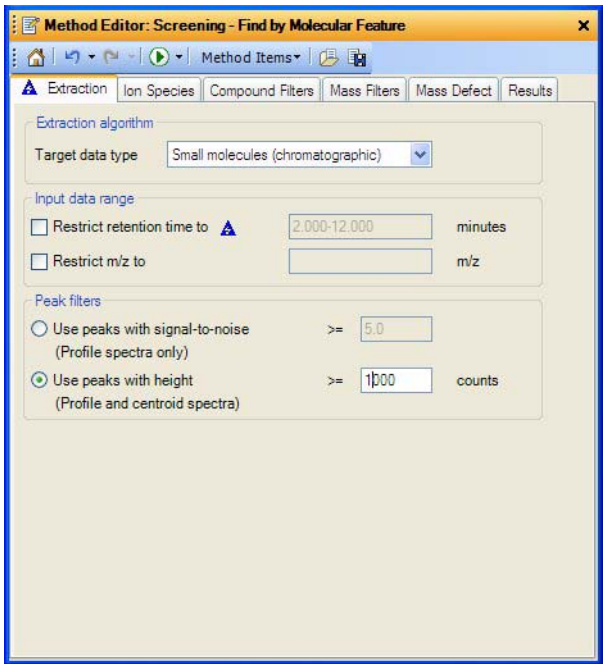


Figure 13 *Find by Molecular Feature – Extraction tab*

Target data type

- Set to **Small molecules (chromatographic)**.

Restrict retention time to

- To reduce computation time, use a smaller window. However, be sure to include the full retention time range where pesticides elute.
- MFE baseline establishment requires 0.3 minutes on either side of the chromatographic peak, so avoid cutting off necessary data.
- Zero is not a valid value, but you can use 0.01.

Restrict m/z to

- To reduce computation time, use a smaller window. However, be sure to include all the *m/z* values you need for pesticides.

Use peaks with height

- This setting is very important. If it is too low, you detect noise and MFE takes a long time to run. If it is too high, the algorithm may fail to detect analytes of interest, or may not detect the isotope peaks that aid with identification.
- A typical setting is about 1000 counts, but it varies with TOF data acquisition parameters and the cleanliness of each system. A setting of up to 5000 may be appropriate. TOF signal (number of counts) is a function of the number of summed transients. A change in acquisition rate changes the number of summed transients, and therefore requires you to adjust this setting.
- In general, set this value to 3X the threshold setting in MassHunter Data Acquisition.
- Or examine spectra to determine the noise level empirically and set the value to 3X the noise level.

To determine the noise level in spectra, do the following;

a Extract the ions of a few pesticides that you analyzed.

b Get the spectra at the top of the peak.

c Look at the noise. For example, expand the y-axis to show 0 to 5000 counts and note the y-axis value of random mass peaks. If they occur around 1000 counts, set **Use peaks with height** to **3000**.

Ion Species tab

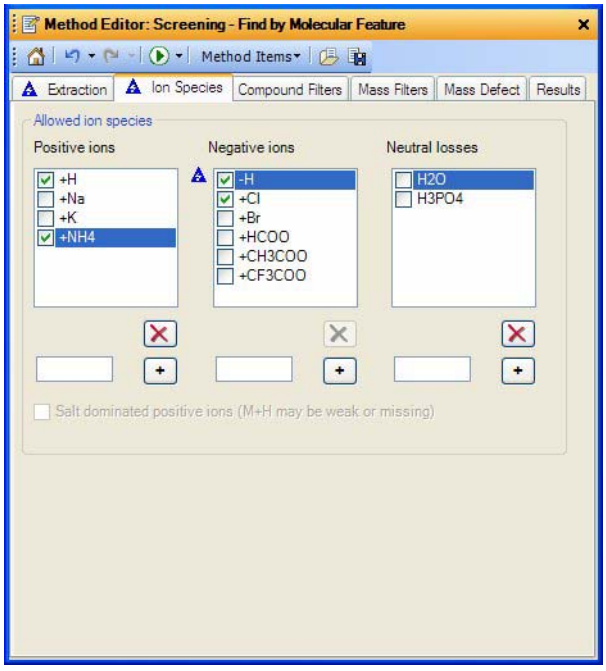


Figure 14 *Find by Molecular Feature – Ion Species tab*

- Mark check boxes for the adduct ions you expect to see. For example, in positive ion mode:
  - If the mobile phase is water/acetonitrile with 0.1% formic acid (or 1% acetic acid), then the expected precursor ion is $[M+H]^+$.

- For some analytes, if the mobile phase modifier is changed to 5 mM ammonium formate (or ammonium acetate), then the precursor may be either $[M+H]^+$ or $[M+NH_4]^+$.
- Sodium ion (Na^+) if present can give a precursor of $[M+Na]^+$.

Compound Filters tab

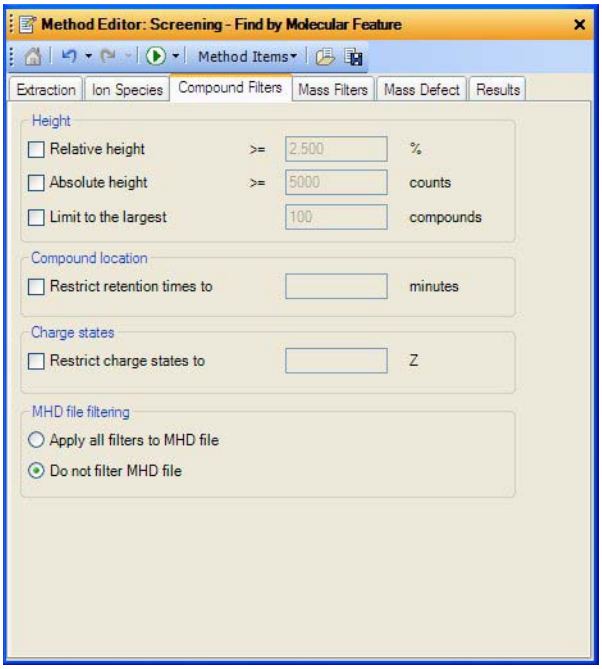


Figure 15 *Find by Molecular Feature – Compound Filters tab*

- Use no filters.

Mass Filters tab

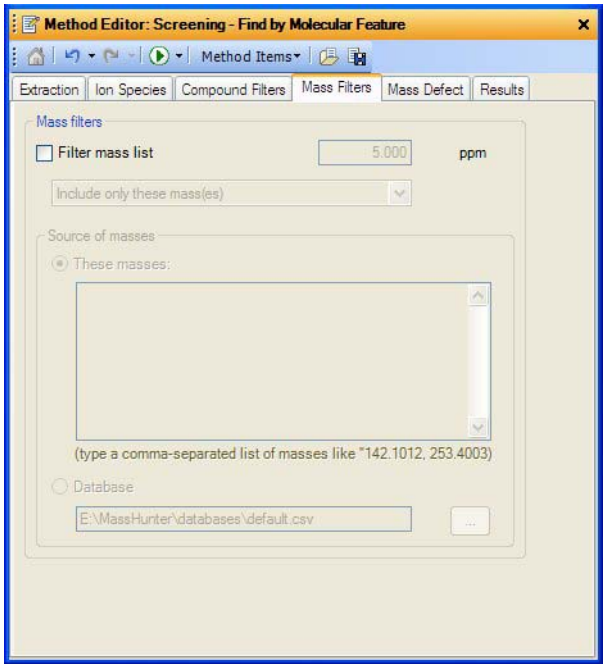


Figure 16 *Find by Molecular Feature – Mass Filters tab*

- Use this filter to include masses of interest. For example, you can include only those in your pesticide database. For details, see step 8 on page 45.
- You can exclude background ions. Make sure that none of the excluded masses are those of pesticides.

Mass Defect tab

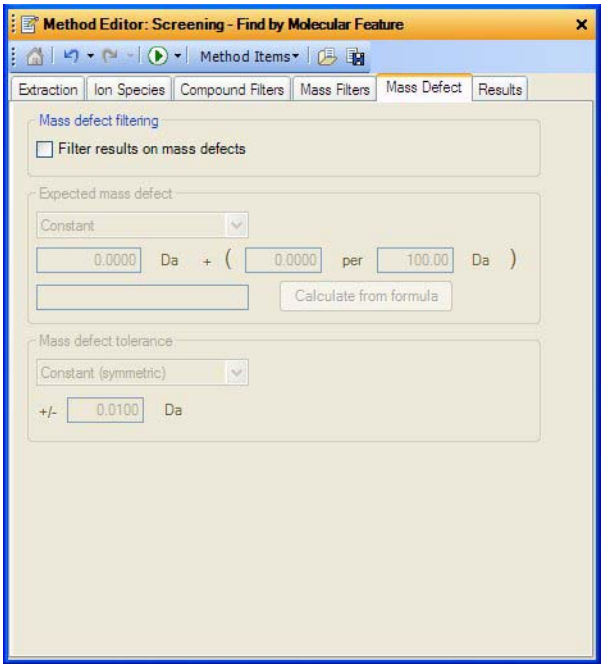


Figure 17 *Find by Molecular Feature – Mass Defect tab*

- Use no filters.

Results tab

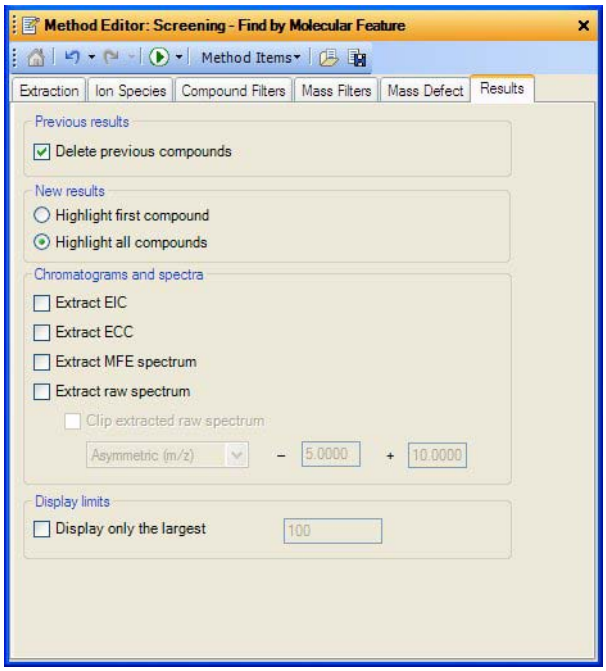


Figure 18 *Find by Molecular Feature – Results tab*

Chromatograms and spectra

- **ECC** – Extracted Compound Chromatogram – is the chromatographic peak as seen by MFE. The ECC shows the isotopes as a merged chromatogram, and includes only the narrow time window when the compound is eluted.
- During method development, you may mark check boxes for **Extract EIC** and **Extract ECC**. After you perform MFE, compare the ECCs and the EICs, particularly for low-abundance peaks. If they look similar, you are likely using appropriate settings for MFE. If they are noisy, you may need to increase the setting (on the **Extraction** tab) for **Use peaks with height**.
- After method development, clear the check boxes for **Extract EIC** and **Extract ECC**, to reduce memory usage. You can always look at results interactively later. To do so, right-click a compound, then click **Extract complete result set.**

Charge State tab

To get here:

a In the Method Explorer, click **Find Compounds > Find by Molecular Feature**.

b Click the **Charge State** tab.

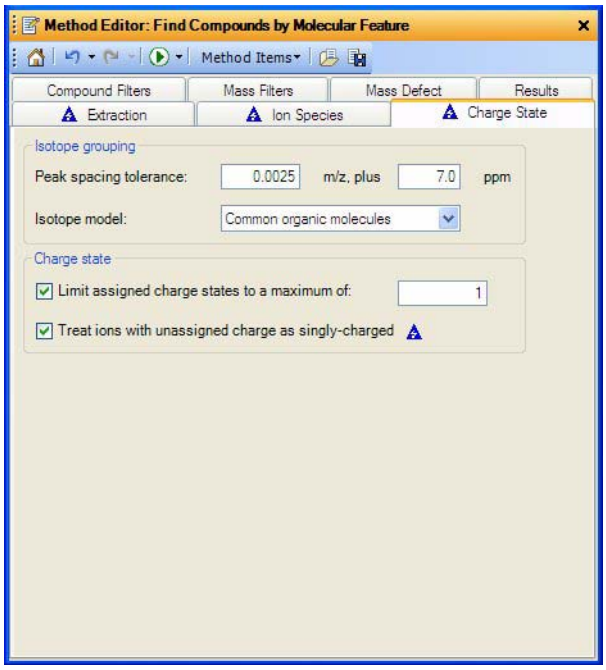


Figure 19 *Find by Molecular Feature – Charge State tab*

Limit assigned charge states to a maximum of

- Set to 1.

Treat ions with unassigned charge as singly-charged

- Mark this check box.

Initial settings for Find by Formula

The following figures show initial settings for Find by Formula. Notes below each figure describe the settings you will likely need to adjust for your analysis.

To view and change these settings, in the Method Explorer, click **MS Target Compound Screening Workflow > Find by Formula**. Then click the appropriate tab.

Formula Source tab

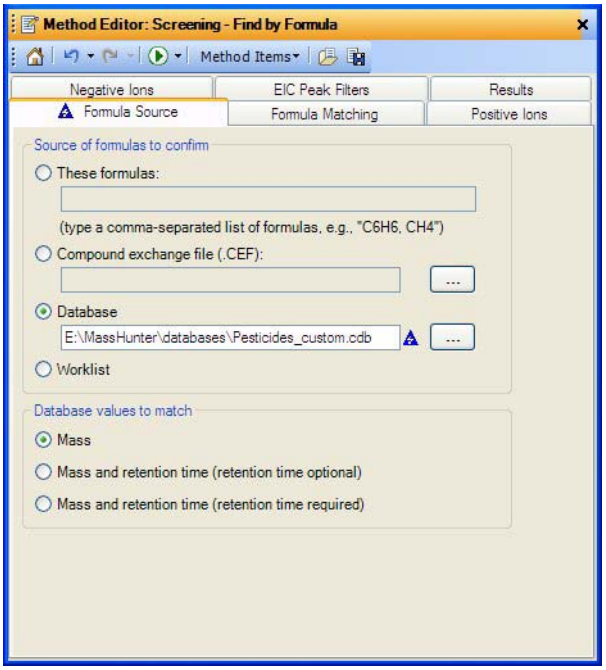


Figure 20 *Find by Formula – Formula Source tab*

Database

- Set to the folder and name of the database you wish to use.

Database values to match

- Click the appropriate option for your analysis. See the online Help for details.

Formula Matching tab

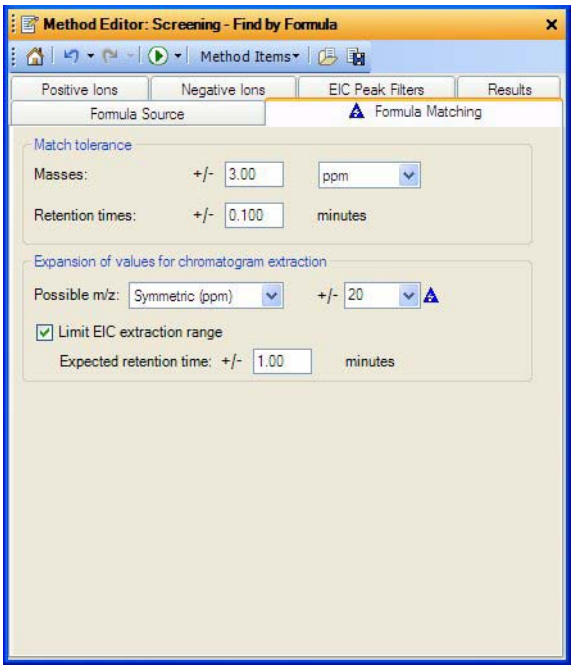


Figure 21 *Find by Formula – Formula Matching tab*

The settings on this tab have a large effect on the number of false positives and false negatives. To ensure results that are of high quality, it is very important to test these parameters with data files that you generate.

Match tolerance

Try the settings in Figure 21 with your data, and adjust if necessary. For example:

- If interfering compounds coelute with some analytes, the mass error increases. Therefore, increase the number for **Masses**.
- If your retention times vary by more than ± 0.1 minutes, increase the number for **Retention times**.

Expansion of values for chromatogram extraction

Try the settings in Figure 21 with your data, and adjust if necessary. For example:

- If interfering compounds coelute with some analytes, the mass error increases. Therefore, increase the number for **Possible m/z**.
- **Expected retention time** determines the window for chromatogram extraction. The setting in Figure 21 is a good starting point. If your retention times are more variable, you may need to increase the window. However, Find by Formula will require more time to extract larger time windows.

NOTE The windows for formula matching are all ± settings. For example, if you set **Retention times** to **0.1** minutes, the program looks for a match within a 0.2-minute window.

Positive Ions tab

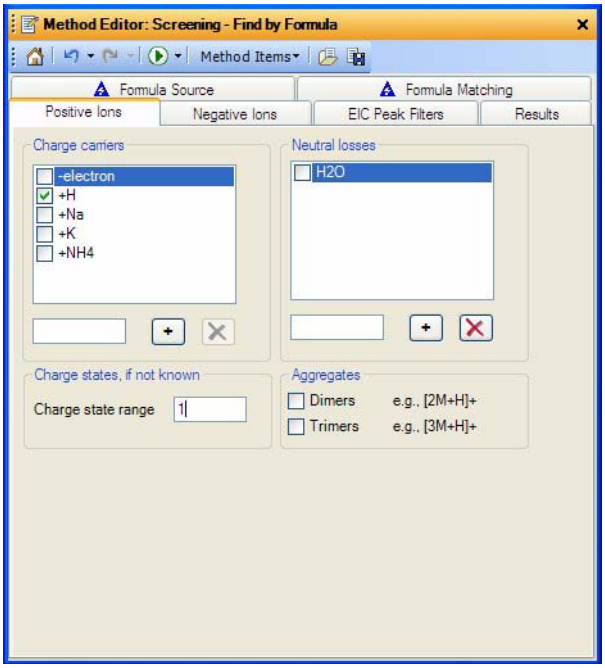


Figure 22 *Find by Formula – Positive Ions tab*

Charge carriers

Mark check boxes for the adduct ions you expect to see. For example:

- If the mobile phase is water/acetonitrile with 0.1% formic acid (or 1% acetic acid), then the expected precursor ion is $[M+H]^+$.
- For some analytes, if the mobile phase modifier is changed to 5 mM ammonium formate (or ammonium acetate), then the precursor may be either $[M+H]^+$ or $[M+NH_4]^+$.

Find by Formula goes faster when you mark fewer adduct ions, so mark only those that are necessary.

Negative Ions tab

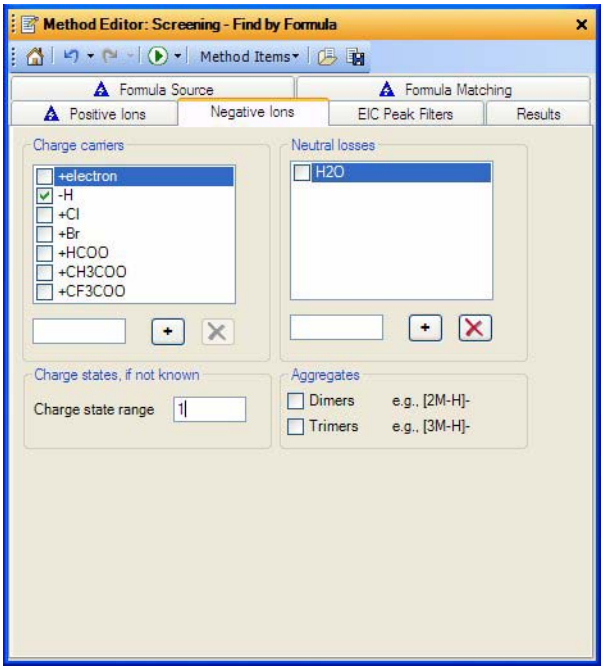


Figure 23 *Find by Formula – Negative Ions tab*

Charge carriers

Mark check boxes for the adduct ions you expect to see. Find by Formula proceeds more quickly when you mark fewer adduct ions, so mark only those that are necessary.

EIC Peak Filters tab

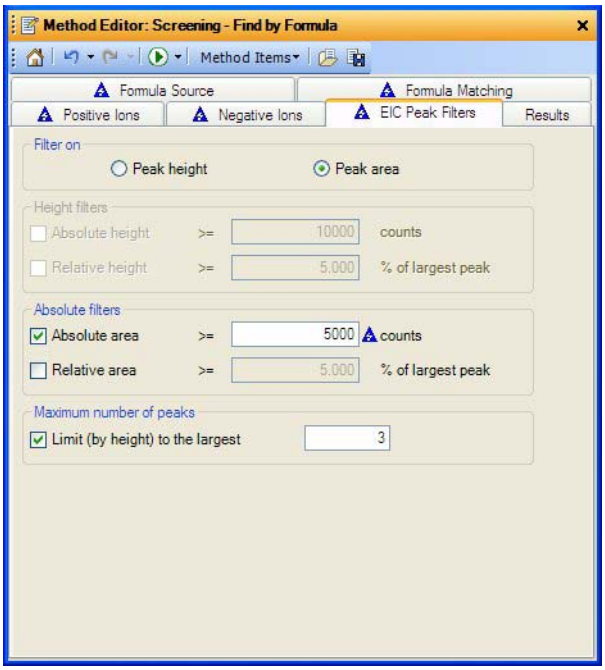


Figure 24 *Find by Formula – EIC Peak Filters tab*

The settings on this tab affect the balance of false positive and false negative results. Be sure to test these parameters with data files that you generate. You want to exclude noise, but not real peaks.

Absolute area

- Examine your data and set to the smallest area you wish to retain from Find by Formula.

Limit (by height) to the largest

- Set to the maximum number of peaks per extracted ion chromatogram (EIC).

Results tab

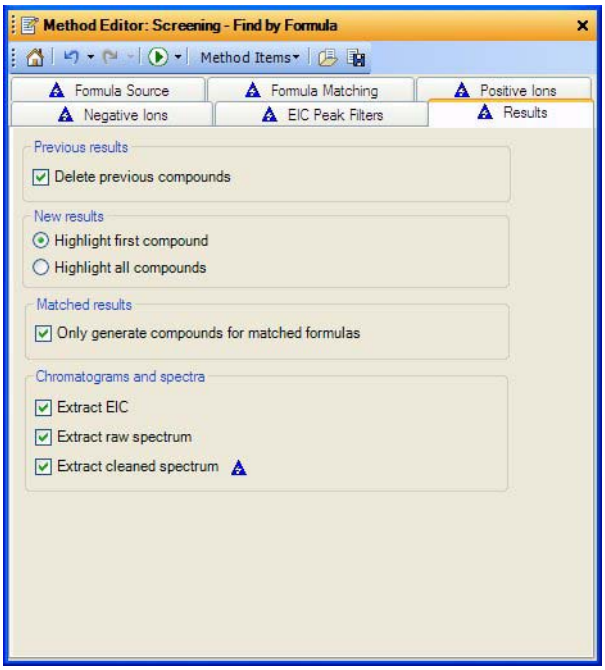


Figure 25 *Find by Formula – Results tab*

Chromatograms and spectra

You may either mark or clear the check boxes. If you clear them, you can later extract the EICs and spectra interactively. Select the compound(s) in either the Compound List or Data Navigator, then right-click and click **Extract Complete Result Set**.

Charge State tab

To get here:

a In the Method Explorer, click **Find Compounds by Formula > Find by Formula – Mass Spectra**.

b Click the **Charge State** tab.

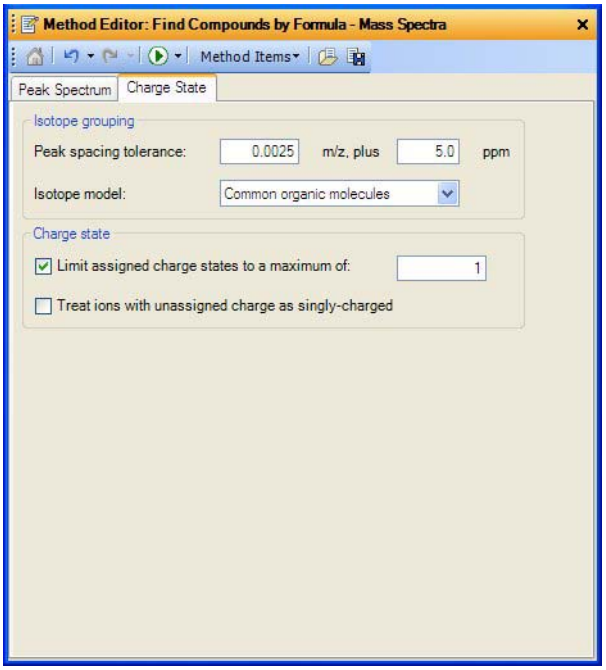


Figure 26 *Find by Formula – Charge State tab*

Limit assigned charge states to a maximum of

- Unless you know you have some pesticides that are doubly charged, set to 1.

Settings to reduce processing time for Find by Formula

Because it uses extracted ion chromatograms, Find by Formula can find pesticides at very low levels. However, processing time can be long if you have MassHunter Qualitative Analysis version B.04 rather than B.05. To reduce processing time you may:

- Create a custom database with only the pesticides of interest, so fewer EICs are generated. For details, see Chapter 3, "Customizing the Personal Pesticide Database," starting on page 23. Specifically, read two sections:
  - "Create a copy of the database" on page 24
  - "Add, delete, and edit database entries" on page 35
- Add retention times to the custom database, so the EICs can use a smaller extraction window. Addition of retention times, described under "Add retention times to the database" on page 26, also reduces false positives.
- Eliminate unnecessary adduct ions, to reduce the number of EICs the algorithm generates.
- Minimize the time range for ion extraction.

To eliminate unnecessary adduct ions:

a In Method Explorer, click **MS Target Compound Screening Workflow > Find by Formula**.

b Click the **Positive Ions** tab or the **Negative Ions** tab (depending on which polarity you plan to run).

c Mark check boxes for only the adducts you expect to see.

To minimize the time range for ion extraction:

a In Method Explorer, click **MS Target Compound Screening Workflow > Find by Formula**.

b Click the **Formula Matching** tab.

c Mark the check box for **Limit EIC extraction range**.

d Set **Expected retention time** to **± 1.0 min**.

References

The references in this list give valuable information that will help you set up multi-residue screening of pesticides with the Agilent 6200 Series Accurate-Mass TOF LC/MS System or the Agilent 6500 Series Accurate-Mass Q-TOF LC/MS System. Many of these documents are available in the online Agilent Literature Library.

Manuals

Agilent G6854AA MassHunter Personal Pesticide Database Kit Quick Start Guide (Agilent publication 5990-4262EN, August 2009)

Agilent 6200 Series TOF and 6500 Series Q-TOF LC/MS System Concepts Guide

Agilent 6200 Series TOF and 6500 Series Q-TOF LC/MS System Quick Start Guide

Agilent 6200 Series Time-of-Flight LC/MS Maintenance Guide

Agilent 6224/6230 Series TOF LC/MS System Maintenance Guide

Agilent 6500 Series Q-TOF LC/MS System Maintenance Guide

Agilent MassHunter Workstation Software – Data Acquisition for 6200 Series TOF and 6500 Series Q-TOF Familiarization Guide (Agilent publication G3335-90066, Third Edition, May 2009)

Agilent MassHunter Workstation Software Qualitative Analysis Familiarization Guide (Agilent publication G3336-90007, Revision B, February 2011)

Agilent G6854 MassHunter Personal Pesticide Database Quick Start Guide (Agilent publication G6854-90003, First Edition, July 2009)

Agilent MassHunter Personal Compound Database and Library Quick Start Guide (Agilent publication G3336-90006, First Edition, July 2009)

Agilent MassHunter Workstation Software Reporting Familiarization Guide (Agilent publication G3335-90110, First Edition, July 2011)

Note: All MassHunter software includes online Help, in addition to manuals. See the online Help for details about the software.

Application and technical notes

"Pesticide Personal Compound Database for Screening and Identification" (Agilent technical note 5990-3976EN, May 2009)

"An Application Kit for Multi-Residue Screening of Pesticides using LC/TOF or Q-TOF with a Pesticide Personal Compound Database" (Agilent application note 5990-4251EN, August 2009)

"Q-TOF LC/MS Screening and Confirming of Non-Targeted Pesticides in a Strawberry Extract" (Agilent application note 5990-3935EN, May 2009)

"Increased Productivity for Target Compound Screening Using TOF-MS and Accurate-Mass Databases with Optional Retention Time – Part 1: Unique Capabilities of Agilent Software" (Agilent technical note 5990-4828EN, December 2009)

"Increased Productivity for Target Compound Screening Using TOF-MS and Accurate-Mass Databases with Optional Retention Time – Part 2: Integrated, Automated Workflows" (Agilent technical note 5990-4829EN, December 2009)

"An Application Kit for the Screening of Samples for Analytes of Forensic and Toxicological Interest using TOF or Q-TOF LC/MS with a Personal Forensics/Toxicology Database" (Agilent application note 5990-4252EN, August 2009)

"Superior Molecular Formula Generation from Accurate-Mass Data" (Agilent technical note 5989-7409EN, January 2008)

"Analysis of Pesticide Residues in Spinach Using Agilent SampliQ QuEChERS AOAC Kit by LC/MS/MS Detection" (Agilent application note 5990-4248EN, August 2009

Other references

"How It Works Video – 6220 Accurate-Mass TOF LC/MS" (available on the Agilent Web site)

"How It Works Video – 6500 Series Accurate-Mass Q-TOF LC/MS Systems" (available on the Agilent Web site)

MassHunter Personal Pesticide Database Kit Support Disk – available with the Agilent MassHunter Personal Pesticide Database Kit (G6854AA) – contains methods

Agilent MassHunter Reporting User Information DVD (Agilent publication G6845-60007, May 2011)

"Innovative Approaches for today's food analysis challenges – Agilent SampliQ QuEChERS Food Safety Applications Notebook," Volume 2, (Agilent publication 5990-4977EN, December, 2009)

"Agilent SampliQ QuEChERS Kits" (Agilent publication number 5990-3562EN, February, 2010)

QuEChERS information is available on the Agilent Web site, including a standard operating procedure and a demo video to help you get started.

www.agilent.com

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